CHEMICAL AND MICROBIAL TRANSFORMATIONS OF SOME STEROIDS AND TERPENES

By

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1961

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
May, 1965

CHEMICAL AND MICROBIAL TRANSFORMATIONS

OF SOME STEROIDS AND TERPENES

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ACKNOWLEDGMENTS

The author is very grateful for the guidance, counsel, and generous research assistantships provided by Dr. Leon H. Zalkow during the course of this study, not to mention his acquisition of funds which made possible the herein documented research.

The author also wishes to express appreciation to Dr. N. N. Durham and Dr. Vera Zalkow for suggestions and assistance during the study, and to those herein unnamed fellow graduate students of Dr. Zalkow's and Dr. Durham's research groups with whom I had the pleasure to work and publish.

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GENERAL INTRODUCTION

Terpenes and steroids are naturally occurring compounds whose microbial and chemical transformations are of interest to chemists and biologists alike. These transformations also provide new compounds of possible pharmacological value. The four chapters of this thesis deal with chemical and microbial transformation of such compounds. The research problems described in the four chapters are not directly related and thus each chapter has its corresponding historical and introduction, results and discussion, and experimental sections.

CHAPTER I

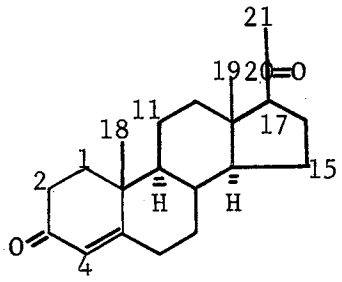
THE MICROBIAL TRANSFORMATION OF PROGESTERONE AND RELATED STEROIDS BY ASPERGILLUS tamarii

Historical and Introduction

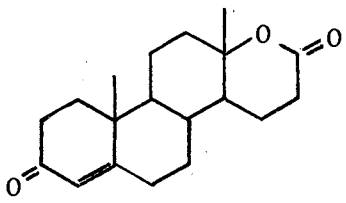
Knowledge concerning the general reaction mechanisms of microbiological conversions of steroids is still very meager. It may be assumed, by analogy to the current concept of enzymatic reactions, that in this case also the attacking enzyme forms intermediate complexes with the steroidal substrates. The shape of the surface of the substrate molecule is thus of primary importance, such that it allows contact at more than one point, as required by the polyaffinity theory. Because of this, a distinct though limited substrate specificity is exhibited. Certain constituents may sterically hinder the microbiological reaction, or at least influence its steric course. Likewise, microorganisms are frequently in a position to carry out reactions which would require many complicated steps in the laboratory.

The only report in the literature concerning the action of the fungi Aspergillus tamarii on steroids appeared in 1955, when Dulany and collaborators (1) reported the transformation of progesterone, I, into 11α-hydroxyprogesterone, VIII, by the fungus. From preliminary reinvestigation of the fermentation of progesterone with Aspergillus tamarii (herein refered to as A. tamarii) it was found that instead of 11α-hydroxyprogesterone other steroidal transformation products were obtained,

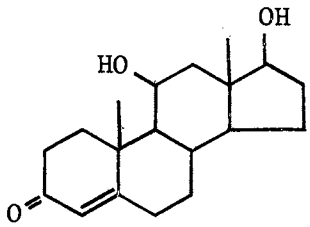
two of which were identified as testololactone, II, and 11β-hydroxytestosterone, III. Chapter I of this thesis describes a study undertaken to determine the nature of the steroidal transformation products of progesterone, I, by A. tamarii and the biosynthetic pathways by which they arose.



I progesterone

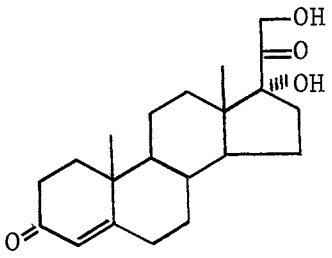


II testololactone

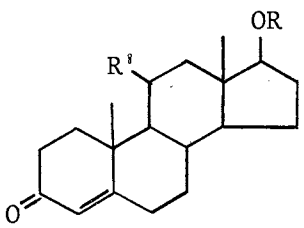


III 11β-hydroxy-testosterone

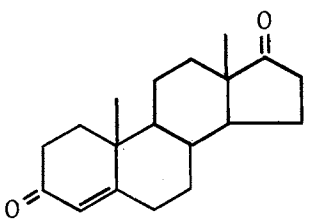
Since the first chemical synthesis of testololactone, II, from progesterone by Levy and Jacobsen (2), several microorganisms have been found which biooxidatively degrade the C-17 β-acetyl side chain with ease to give the corresponsing D ring lactone. In 1953, Peterson and co-workers (3) reported the utilization of Aspergillus flavus and Penicillium adametzi in the conversion of progesterone and 17α-hydroxyprogesterone to testololactone. In the same year Fried, et al. (4) reported that fermentation of progesterone with several fungi of the genera Aspergillus and Mucor, as well as Penicillium crysogenum result in formation of testololactone. Biooxidation involving both lactone formation in the D ring and dehydrogenation in the A ring was also observed by Fried and co-workers (4). Thus fermentation of progesterone, Reichstein's compound S, IV, or testosterone, V, with Cylindrocapon radicola gave Δ^1-dehydrotestololactone.



IV Reichstein's S

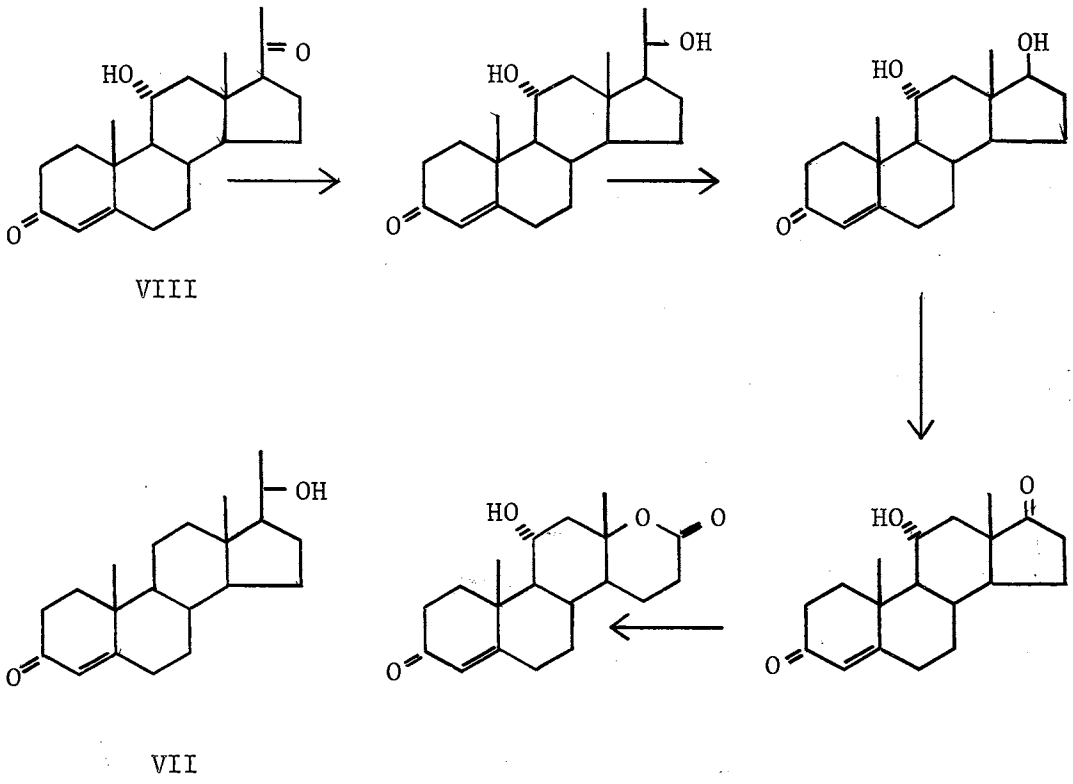


V(R=R'=H) testosterone
IX(R=$COCH_3$; R'=H)
X(R=H; R'=OH)

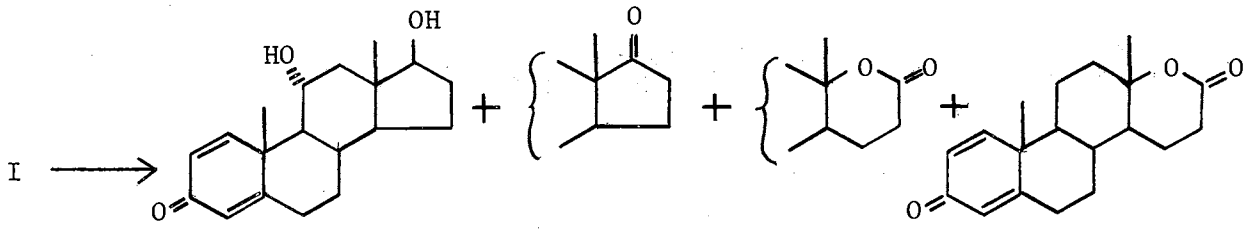


VI Δ^4-androstene-3,17-dione (androstandione)

Since these first communications, other workers (5-8) have reported formation of testololactone from progesterone or progesterone derivatives. In recent years microbiological transformation of the C-17 β-acetyl side chain of progesterone to testololactone has been subjected to mechanistic study. Capek et al. (5) found that progesterone was predominantly transformed in succession into testosterone, Δ^4-androstene-3,17-dione, and finally testololactone. More recently, Sebek et al. (9) investigated the progesterone metabolism by Penicillium titacinum in detail and identified 20β-hydroxy-4-pregnen-3-one, VII, in the early stages of the conversion. Sebek et al. (9) also showed that similar biooxidation of 11α-hydroxyprogesterone, VIII, gave the 11α-hydroxylanalogues of the progesterone transformation intermediates found by Capek et al. (5), and arrived at the following scheme.

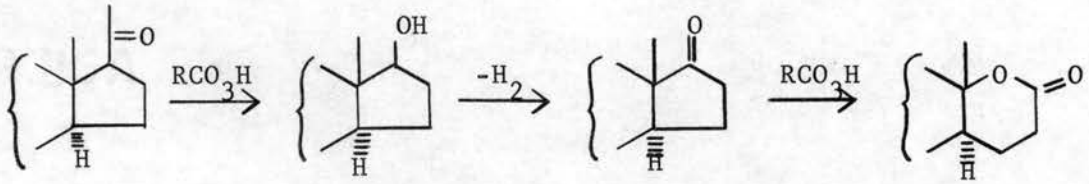


Similarly, Fried (10) has reported finding analogous intermediates in the conversion of progesterone with Fusarium javanicum as shown below.



It is possible that other pathways exist for the initial acetyl side chain cleavage. In 1960, Fonken et al. (11) identified testosterone acetate, IX, as a result of the side chain cleavage of progesterone by Cladosporium resinae, together with androstendione, VI, and

testosterone, V. When ^{14}C-21 labeled progesterone was used as the fermentation substrate, the entire radioactivity was found in the testosterone acetate after the fermentation. A similar pathway was suggested in 1953, by Fried et al. (4), and proposed even earlier (12, 13) for the degradation of 20-oxosteroids by peracids, which likewise proceeds with retention of configuration at C-17.



Still another pathway involving initial C-17 hydroxylation has been proposed by Kowal et al. (14).

Results and Discussion

We (15) have found that A. tamarii transforms progesterone by two independent paths. The major pathway leads to the end-product testololactone, II, which is produced at an early stage via the intermediates testosterone, V, and Δ^4-androstene-3,17-dione, VI, as previously postulated (5, 9). The second path leads to the end-product 11β-hydroxytestosterone, X. These findings differ from the previous report (1) of steroidal transformations by A. tamarii which noted the 11α-hydroxylation of progesterone. However, the A. tamarii (16) used in this study was recently isolated from the soil which might account for the different fermentation paths.

Thin-layer chromatography of the crude product obtained after incubation of progesterone with A. tamarii for 48 hours showed the presence of three compounds (R_f 0.72, 0.25, and 0.09). Preparative chromatography on alumina provided the three compounds in pure form and showed that the

least polar substance (R_f 0.72) was unreacted progesterone (13%), whereas the product with R_f 0.25 was testololactone (70%) and the most polar product (R_f 0.09) was 11β-hydroxytestosterone (14%). When the incubation was interrupted after 12 hours, the thin-layer chromatogram of the crude product showed four compounds (R_f 0.95, 0.72, 0.42, and 0.25) identified as Δ^4-androstene-3,17-dione, VI, progesterone, I, testosterone, V, and testololactone, II, respectively. After 24 hours of incubation, the same four compounds were present but the relative amount of progesterone had decreased, whereas the amount of testololactone had greatly increased and Δ^4-androstene-3,17-dione and testosterone were present in small amounts. When Δ^4-androstene-3,17-dione, VI, was incubated with A. tamarii for 48 hours, testololactone was the only product evident at this time. Incubation of testosterone, V, under similar conditions gave 11β-hydroxytestosterone, X, in 25% yield and testololactone, II, in 41% yield and unchanged testosterone in 31% yield. Testosterone is apparently an intermediate in the formation of both II and X. When 11β and 11α-hydroxyprogesterone were incubated with A. tamarii the corresponding 11-hydroxytestosterones were produced but none of the corresponding testololactones were formed.

Thus it appears that A. tamarii is not capable of oxidatively cleaving the D ring of 11-hydroxylated progesterones and testosterones. Δ^4-Androsten-11β-ol-3,17-dione was unchanged by A. tamarii thus indicating that the oxidative cleavage of the D ring of the 11-hydroxylated steroids is not inhibited solely by the lack of enzymes capable of oxidizing the 17-hydroxyl group to a keto group. The increased relative yield of 11α-hydroxytestosterone in the incubation of testosterone compared to incubation of progesterone, suggests that in the latter case the

intermediate testosterone is not completely free and the "bound" testosterone is preferentially transformed into testololactone. When testosterone itself is incubated with A. tamarii, the enzyme system which converts it to 11β-hydroxytestosterone successfully competes for it with the enzyme system which converts it to testololactone.

Sebek et al. (9) found that 11α-hydroxyprogesterone was converted into 11α-hydroxytestololactone by Pencillium lilacinum and Fried (10) found that Fusarium javanicum transformed progesterone into 11α-hydroxy-testololactone and a number of other products. Thus, A. tamarii appears unusual in its inability to oxidatively cleave the D ring of 11-hydroxylated steroids.

Experimental

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Analyses were performed by Midwest Microlab, Inc., Indianapolis, Indiana. Infrared spectra were recorded with a Beckman IR-5 spectrometer and n.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, using tetramethylsilane as an internal standard ($\delta = 0$). Thin-layer chromatograms (TLC) were run on 35-μ-thick Silica Gel G-coated glass plates using ethyl acetate as the mobile phase; iodine vapors were used for detection; R_f's were reproducible within ± 0.03.

General Methods

Fermentation. A. tamarii (16) spores (maintained on Sabouraud agar slants) were inoculated aseptically into 250 cc. flasks containing 100 cc. of 3% malt extract medium (Difco). After a 48-hour incubation period at 25° C on a rotary shaker, 50 mg. of the steroid, dissolved

in 0.4 cc. of dimethyl formamide, was added to each flask and the incubation continued for 48 hours at 25° C with shaking.

Extraction. The flask contents were combined, the mycelium filtered, washed with chloroform and the washings added to the filtrate. The filtrate was extracted by stirring with a volume of chloroform equal to one-half its volume for 24 hours. The chloroform layer was removed and the extraction process repeated. The combined chloroform extracts were dried over anhydrous magnesium sulfate and concentrated with a rotary evaporator and finally under vacuum. The solvent-free extracts were often crystalline.

Progesterone Transformation by A. tamarii.

Progesterone (17) (1.2 g.) was added to 2400 cc. of a 48-hour growth of A. tamarii as described above. After 48 hours of incubation, chloroform extraction gave 1.03 g. of crude crystalline transformation product. TLC showed 3 spots with R_f's 0.72, 0.25, and 0.09. The crude extract was chromatographed on 70 g. of Merck acid-washed alumina (activity III). The ether-benzene (1:9) eluent, gave 128 mg. of amorphous solid which showed only one spot in TLC; the melting point (130-131° alone and admixture) and infrared and n.m.r. spectra were identical with those of authentic progesterone.

Further ether-benzene (4:6) eluent gave 722 mg. of testololactone, m.p. 207-209° [reported (1) m.p. 205.5-207°]. TLC of the eluent showed one spot, R_f 0.25. υ^{KBr} 1720, 1670, 1620 cm^{-1}. N.m.r. ($CDCl_3$): 1.17 δ (3 protons), 1.35 δ (3), 5.75 δ (1). The infrared and n.m.r. spectra were superimposable on those of an authentic sample (16).

The methanol-ether (1:99) eluent gave 139 mg. of 11β-hydroxytestosterone. TLC of the eluent showed only one spot, R_f 0.09.

Recrystallization from 1:1 methanol-ether gave, after drying at 0.1 mm Hg, m.p. 241-243° [reported (18) m.p. 241°]. $[\alpha]_D^{23}$ 142° (c, 0.0164 in $CHCl_3$) [reported (18) $[\alpha]_D^{24}$ 159°] ν^{KBr} 3500, 1670, 1620 cm^{-1}. N.m.r. ($CDCl_3$): 1.03 δ (3), 1.45 δ (3), 2.10 δ (1), 2.34 δ (1), 3.56 δ (1), 5.67 δ (1).

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.97%; H, 9.27%.

Found: C, 74.88%; H, 9.40%.

Oxidation of 11β-Hydroxytestosterone to Δ^4-Androstene-3,11,17-trione.

To 20 mg. of 11β-hydroxytestosterone in 3 cc. of dry pyridine was added dropwise a solution of 100 mg. of CrO_3 in 2 cc. of pyridine. After stirring at room temperature for 10 min. the pyridine was removed under vacuum and the dark-colored residue poured into 200 cc. of water. The resulting solution was extracted with three-20 cc. portions of ether. The combined ether extracts were dried over magnesium sulfate and removal of the solvent gave 17 mg. of Δ^4-androstene-3,11,17-trione, m.p. 219-220° after recrystallization from methanol [reported (19) 220-223°]. ν^{KBr} 1740, 1710, 1670, 1620 cm^{-1}.

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.96%; H, 8.05%.

Found: C, 76.01%; H, 8.32%.

This compound was identical in all respects with an authentic sample.

Progesterone Conversion after 12, 24, and 48 Hours on Incubation.

To each of twenty-four 250 cc. flasks containing 100 cc. of a 48-hour growth of A. tamarii was added 50 mg. of progesterone. Eight of the flasks were incubated for 12 hours, eight for 24 hours, and eight for 48 hours. At each time interval the contents of the eight flasks were pooled, chloroform extracted, and the crude steroidal metabolite isolated as previously described. The extracts from the 12, 24, and 48

hour incubations were analyzed by TLC. TLC of the 12-hours incubation product showed four compounds identified as progesterone (R_f 0.72), androstenedione (R_f 0.95), tesosterone (R_f 0.42) and testololactone (R_f 0.24) by comparison with authentic samples. The relative intensity of the iodine-detected spots showed progesterone to be present in large amount. TLC of the 24-hour incubation product showed the same four spots but the relative amount of progesterone had decreased, androstenedione and testosterone appeared only in small amounts, and testololactone had increased significantly. TLC of the 48-hour incubation product showed the presence only of progesterone, testololactone and 11β-hydroxytestosterone (R_f 0.09). Androstenedione and testosterone had completely disappeared.

Conversion of 11α-Hydroxyprogesterone (17) by A. tamarii.

11α-Hydroxyprogesterone (1.20 g.) was incubated with A. tamarii for 48 hours as previously described and 1.08 g. of crude amorphous product was obtained. TLC showed two spots with R_f's 0.37 and 0.20. The crude chloroform extract was chromatographed on 70 g. of Merck acid-washed alumina (activity III). The ether-benzene (1:9) eluent gave 157 mg. of 11α-hydroxyprogesterone (R_f 0.37), m.p. 169-172° alone and on admixture with an authentic sample. υ^{KBr} 3450, 1710, 1670, 1620, 870 cm^{-1}. The ether eluent gave 655 mg. of 11α-hydroxytestosterone, m.p. 181-181.5°, after recrystallization from ether [reported (20) m.p. 181.5°], R_f 0.20. υ^{KBr} 3450, 3320, 1660, 1620, 865 cm^{-1}. $[\alpha]_D^{26}$ 94°, c, 0.0284 in $CHCl_3$ [reported (20) [α] 93°]. N.m.r. ($CDCl_3$): 1.83δ (3), 1.32δ (3), 2.20δ (1) which disappeared upon addition of D_2O, 2.29δ (1), 2.35δ (1), 5.74δ (1). None of the chromatography fractions showed 6-membered lactone absorption, indicative of 11α-hydroxytestololactone, in the infrared.

Conversion of 11β-Hydroxyprogesterone by A. tamarii.

11β-Hydroxyprogesterone (167 mg.) was incubated with A. tamarii for 48 hours as previously described. The usual work-up gave 145 mg. of crude product, whose TLC showed only two spots, R_f 0.35 and R_f 0.09. The crude extract was chromatographed on 35 g. of Merck acid-washed alumina (activity III) and the ether-benzene (3:7) eluent gave 87 mg. of unchanged 11β-hydroxyprogesterone, m.p. 185-187°, R_f 0.35, identical in all respects with authentic material. The ether-benzene (8:2) eluent afforded 46 mg. of 11β-hydroxyprogesterone (R_f 0.09), m.p. 237-240°. υ^{KBr} 3500, 1670, 1620 cm^{-1}. This material was identical in all respects with 11β-hydroxytestosterone obtained as described above. None of the chromatography fractions showed the presence of 6-membered lactones in their infrared spectra.

Transformation of Δ^4-Androstene-3,17-dione by A. tamarii.

Δ^4-Androstene-3,17-dione (332 mg.) was incubated with A. tamarii for 48 hours as previously described and chloroform extraction gave 235 mg. of crude product. TLC showed only two spots (R_f 0.96 and 0.25). The crude extract was chromatographed on 65 g. of Merck acid-washed alumina (activity III). The ether-benzene (1:9) eluent gave 167 mg. of unchanged Δ^4-androstene-3,17-dione, m.p. 169-172°, R_f 0.96, υ^{KBr} 1735, 1670, 1620 cm^{-1}. The ether-benzene (6:4) eluent gave 66 mg. of testololactone (R_f 0.25).

Conversion of Testosterone by A. tamarii.

Testosterone (1.00 g.) was incubated with A. tamarii for 48 hours as previously described and the usual work-up gave 877 mg. of crude product, the TLC of which showed three spots; R_f 0.43, 0.25, and 0.09. The crude extract was chromatographed on 20 g. of Merck acid-washed

alumina (activity IV); the benzene eluent gave 624 mg. of non-crystalline material, the TLC of which showed two spots of R_f 0.43 and R_f 0.25, corresponding respectively to testosterone and testololactone. Recrystallization of the combined chromatography fractions from benzene, gave after drying at 0.1 mm Hg, 210 mg. of pure testosterone, m.p. 149-151°. Rechromatography of the residue, from the recrystallization, on 35 g. of Merck acid-washed alumina (activity IV) gave 363 mg. of pure testololactone, m.p. 205-207°, R_f 0.25.

The ether-benzene (8:2) eluent, gave 217 mg. of 11β-hydroxytestosterone, m.p. 238-240°, R_f 0.09, identical in all respects with that obtained as previously described.

CHAPTER II

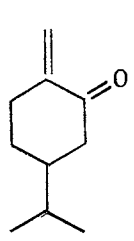
THE OCCURRENCE OF THE SANTOLINENONES

Historical and Introduction

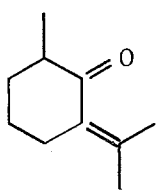
Santolina chamaecyparissus L. (family Compositae), the so-called "Cypress Lavender-Cotton," is an evergreen subshrub native to Southern Europe possessing a strong and penetrating aromatic odor. Because of its antispasmotic and anthelmintic properties, the plant was formerly in the pharmacopoeias of various countries, and even today is used in old-fashion medicines in Europe.

The steam-volatile essential oil from Santolina chamaecyparissus L. has been shown by Francesconi and collaborators (21, 22) to contain three ketones, $C_{10}H_{16}O$, for two of which structures XI (α-santolinenone) and XII (β-santolinenone) were suggested. These ketones were not obtained in pure form and the structures assigned to them must be regarded as doubtful (23-26) since they were deduced solely from a study of their derivatives.

Chapter II of this thesis documents a reinvestigation of the composition of the steam-volatile essential oil of Santolina chamaecyparissus L.



XI



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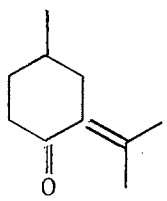
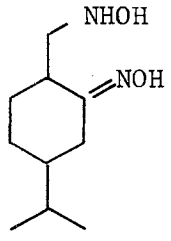


XII

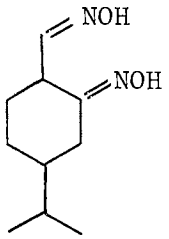


XIII

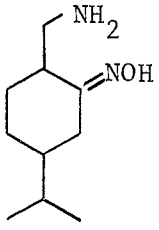
Francesconi and Scorafia (21) reported that when an alcoholic solution of the oil from S. chamaecyparissus was treated with hydroxyl amine hydrochloride in the presence of sodium carbonate, a mixture of products was obtained; a hydroxlamin-oxime, two liquid oximes and a hydroxylamine. The hydroxylamine-oxime could be separated either by direct crystallization or by steam distillation. In the latter case the other products were volatile. α-Santolinenone hydroxylamino-oxime (27) crystallized in prisms, m.p. 190°, but it could not be hydrolyzed to the parent ketone, since on treatment with mineral acids it behaved like pulegone hydroxylamino-oxime and gave an iminonitrile (28).

The formation of the hydroxylamino-oxime indicated that the carbonyl group and the olefinic linkage must be conjugated, and Francesconi and Scarafia (21) suggested that the ketone could be represented most satisfactorily by structure XI, and the hydroxylamino-oxime by structure XIII.

When the hydroxylamino-oxime, XIII, was heated in alcoholic solution simultaneous oxidation and reduction took place with a formation of a dioxime of postulated structure XIV, m.p. 260°, and an amino-oxime postulated as XV, m.p. 155°.



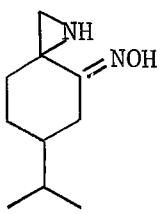
XIV



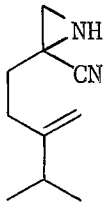
XV

By the action of mineral acids on the hydroxylamino-oxime, XIII, α-santolinenone-imino-oxime, XVI, m.p. 169-172°, and α-santolinenone nitrile imine, XVII, m.p. 119-120°, were reported together with

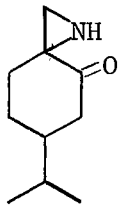
α-santolinenone imine, XVIII. These structures were only suggested by Francesconi and collaborators and have not been confirmed. In fact, these structures are very doubtful.



XVI



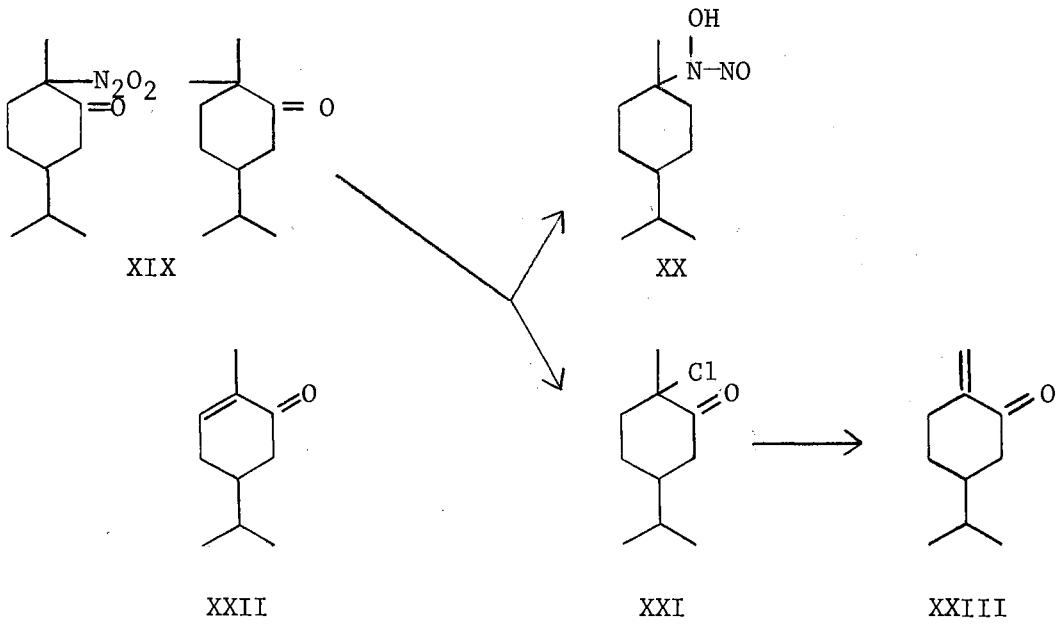
XVII



XVIII

Although the suggested structures for α-santolinenone contains an asymmetric carbon atom, the various derivatives mentioned above were found to be optically inactive. Francesconi and Granata (29) postulated that the ketone was obtained as a racemate, since they reported the resolution of its hydroxylamino-oxime by means of d-camphor-β-sulfonic acid.

If the structure attributed to α-santolinenone (XI) was correct, then it should have been identical with the ketone obtained by Breyer and Oehler (39) on treatment of l-bis-nitroso-carvomenthone, XIX, with hydrogen chloride to yield XX and XXI, followed by treatment of the latter with sodium acetate to yield XXIII. The latter was found not to be identical and carvatan-acetate, XXII, and apparently α-santolinenone and its derivatives were never compared with XXIII.



XIX XX XXII XXI XXIII

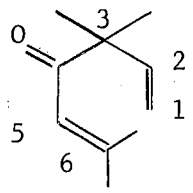
As was noted above, one of the products formed by the action of hydroxylamine hydrochloride on the oil from S. chamaecyparissus was a hydroxylamine, designated by Francesconi and Granata (22) as β-santolinenone hydroxylamine. The hydroxylamine, m.p. 62-64°, was stable and could not be hydrolyzed to the parent ketone. On oxidation with mercuric oxide, it gave a nitroso-compound, m.p. 60-62°, which became blue when fused or placed in a solution.

According to Francesconi and Granata (22), β-santolinenone is best represented by structures XII. It is, however, improbable that these structures are correct (26).

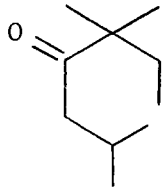
Very little appears in the literature concerning the nature of saturated $C_{10}H_{16}O$ ketone, γ-santolinenone, except that it is probably of the camphor type (31).

Results and Discussion

Reinvestigation (32) of the steam-volatile oil of S. chamaeparissus L. using gas-chromatography showed it to contain one major (65%) and three minor components. Preparative gas chromatography accomplished the separation of the four components and the major component has been shown to be 3,3,6-trimethyl-1,5-heptadiene-4-one, XXIV, a monoterpene which does not obey the "isoprene rule" (33). It is this compound which is responsible for the strong penetrating odor of the plant.



XXIV



XXV

The infrared spectrum of XXIV showed the presence of an α,β-unsaturated carbonyl band at 1670 and a strong band at 1635 cm^{-1}. The n.m.r. spectrum of XXIV showed a strong six-proton singlet at δ 1.18 (two methyl groups at C-3) and two three-proton singlets at δ 1.89 and δ 2.10 (two methyl groups at C-6); the vinylic region showed a typical ABX splitting pattern with the X proton (proton attached to C-2) giving a quartet centered at δ 5.94 with J_{AX} = 10 c.p.s. and J_{BX} = 18 c.p.s. and the AB protons (protons attached to C-1) appearing as an octet in the range δ 4.91-6.20 with J_{AB} = 1.5 c.p.s.; the C-5 proton gave a broad signal centered at δ 6.18.

The elemental analysis of XXIV, together with its g.l.c. retention time and n.m.r. spectrum indicated the molecular formula to be $C_{10}H_{16}O$,

and the mass spectrum of XXIV confirmed this by showing the molecular ion peak at mass 152. As shown in Plate I, page 70, the mass spectral splitting pattern of XXIV, the most intense peak in the spectrum (39.4% of total ion yield) appeared at mass 83 and another intense peak appeared at mass 55. Both of these peaks arise from cleavage α to the carbonyl, a process frequently encountered with saturated ketones (34).

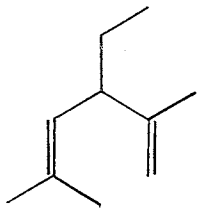
Chemical evidence for structure XXIV was obtained by its hydrogenation to 3,3,6-trimethylheptan-4-one, XXV, which itself was synthesized by a procedure similar to that previously described (35). N.m.r. and mass spectral analysis (see Plate 2) confirmed structure XXV.

Ketone XXIV had been previously isolated from Artemesia annua L. (36). There is confusion in the literature regarding the name given to ketone XXIV isolated from Artemesia annua L. In some cases it is called artemesia ketone and in other cases it is referred to as isoartemesia ketone and likewise the isomeric ketone, 3,3,6-trimethyl-1,6-heptadiene-4-one, also isolated from Artemesia annua L., is referred to by one or the other of these names.

The presence of any substances of structure XI (α-santolinenone) or XII (β-santolinenone) could not be detected in the oil examined. It thus seems unlikely that α- or β-santolinenones have been isolated from naturally occurring substances.

The structure of the major component of the steam-volatile oil of S. chamaecyparissus L. and the dubiety of the existence of the "santolinenones" has been confirmed by Thomas and Willhalm (37) of the Research Laboratories, Firmenich et Cie, Geneve, Switzerland. Besides ketone XXIV they also identified myrcene (8%) and detected α-pinene in the leaves of the plant. Examination of the steam-volatile oil obtained

from the flowers of the same plant gave the Swiss group of much more complex mixture than the oil examined from the whole plant. Compounds positively identified (by infrared and mass spec) were α-thujene, α- and β-pinene, β-phellandrene, myrcene, limonene, p-cymene, ar-curcumene, acetone, cryptone, menth-1-en-4-ol, and α-terpineol. They also isolated a new hydrocarbon which they believe to have structure XXVI.



XXVI

Experimental

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR-5 spectrophotometer; n.m.r. spectra were determined with the Varian A-60 spectrometer using carbon tetrachloride as solvent and tetramethylsilane as an internal standard ($\delta = 0$). Preparative gas chromatography was accomplished with the Aerograph A-700 Autoprep using an 0.5-in.-diameter by 20-ft. column of 20% Ucon Polar on Chromosorb W, a column temperature of 150°, and a helium flow rate of 200 cc. per min. Analytical gas chromatographs were obtained with Aerograph's Hy-Fi and Model A-350B gas chromatographs. Mass spectra were obtained with a Consolidated Electrodynamics Corporation Model 21-103C mass spectrometer (Continental Oil Company, Ponca City, Oklahoma).

Steam Distillation of Santolina chamaecyparissus L.

The leaves and stems of the plant were collected in early winter

from the Stillwater, Oklahoma, gardens of the Department of Horticulture, Oklahoma State University, Stillwater, Oklahoma, (38). The coarsely chopped, air-dried plant material (800 g.) was steam distilled for 2 hrs. to give 3.8 l. of distillate, which was extracted with 500 cc. of ether. After drying over magnesium sulfate, the ether was removed with a 45-in. Todd fractionation column at 40°, leaving a residue of 5.74 g. (0.75% based on dry plant) of light yellow oil. η_D^{25} 1.4705. υ_{max}^{film} 1635, 1775, 1750, 1675, 1630, and 880 cm^{-1}. Attempted column chromatography of the oil on neutral alumina resulted in recovery of only a small amount of material. The infrared spectrum of this material did not compare with the spectrum of the crude oil, thus indicating the instability of the oil to chromatography on alumina. Analytical gas chromatography of the oil using the Aerograph Hy-Fi hydrogen flame detector, and the Model A-350B gas chromatographs with several columns indicated the crude oil contained predominantly one major and three minor components.

Isolation of XXIV.

Preparative gas chromatography of the oil utilizing the Aerograph A-700 Autoprep using a 0.5-in.-diameter by 20-ft. column of 20% Ucon Polar on Chromosorb W, a column temperature of 150°, and a helium flow rate of 200 cc. per min. gave four components with the retention time, yields, and properties shown below.

Component A had a retention time of 2.4 min., yield 4%, $\eta_D^{26.5}$ 1.4811. This compound was too volatile to further characterize, however, infrared analysis indicated it to be a hydrocarbon.

Component B had a retention time of 25.6 min., yield 15%, $\eta_D^{26.5}$ 1.4679; infrared spectrum showed bands at υ_{max}^{film} 1650, 1600, and 895 cm^{-1}; n.m.r. showed a quartet (J = 11 c.p.s.) centered at δ 6.30, a strong

signal at δ 4.95, several weaker signals in the region of δ 4.5-5.3, and strong, sharp signals at δ 0.72, 1.24, 1.60, 1.69 and 2.18.

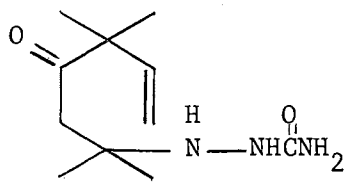
Component C had a retention time of 37.5 min., yield 10%, $\eta_D^{26.5}$ 1.4679; infrared spectrum showed bands at υ_{max}^{film} 1742, 1650, 1600, and 880 cm^{-1}.; n.m.r. showed a quartet (J = 11 c.p.s.) centered at δ 5.87 and sharp signals at δ 4.66, 0.95 and 0.87.

3,3,6-Trimethyl-1,5-heptadiene-4-one, XXIV, had a retention time of 50.1 min., yield 65%, η_D^{25} 1.4670 [reported (39) η_D^{25} 1.4631], b.p. 180-182° [reported (39) b.p. 182°]; ultraviolet spectrum using a Beckman DK-1 showed λ_{max}^{alc} 238 mμ (ε 11,275); infrared showed υ_{max}^{film} 1675, 1635, 910, and 890 cm^{-1}. For n.m.r. see Results and Discussion section above. For mass spectrum see Plate I.

Anal. Calcd. for $C_{10}H_{16}O$: C, 78.89%; H, 10.60.

Found: C, 78.51%; H, 10.71%.

The semicarbazide adduct, XXVII, (addition of semicarbazone across the conjugated double bond) was prepared as previously described (39), m.p. 71-72° [reported (39) m.p. 68-75°].



XXVII

Preparation of 3,3,6-Trimethylheptan-4-one.

When 0.74 g. of ketone XXIV was hydrogenated in 15 cc. of methanol in the presence of 0.08 g. of 10% palladium on carbon at room temperature and atmospheric pressure, 218 cc. (1.98 molar equivalents) of hydrogen

were rapidly absorbed. The catalyst was removed by filtration, and 50 cc. of water was added to the methanolic filtrate. The aqueous methanol solution was extracted with ether and, after drying over anhydrous magnesium sulfate, the ether was removed with a fractionation column. The residue was distilled to give ketone XXV, b.p. 70-75° (25 mm), $\eta_D^{25.2}$ 1.4245; infrared showed υ_{max}^{film} 1715 cm^{-1}; n.m.r. showed δ 0.85 (doublet, J = 7 c.p.s.), 0.90 (doublet, J = 7 c.p.s.), and 1.07 (six-proton signlet). Gas chromatography on a 1/8-in. by 10-ft. 20% Ucon Polar on Chromosorb W column using a hydrogen flame detector showed XXV to be homogeneous. See Plate II for mass spectrum.

Ketone XXV was synthesized by a procedure similar to that used by Colonge and Dumont (35) for the synthesis of XXIV. 2,2-Dimethylbutyrolyl-chloride (2.5 g.), prepared by the reaction of 2,2-dimethylbutyric acid with thionyl chloride, was added to a stirred suspension of 1.0 g. aluminum chloride in 60 cc. of dry chloroform at 0°. Dry isobutylene was passed slowly through this suspension for 3 hr. and, after filtration, the solution was washed with a cold aqueous sodium hydroxide solution. The organic layer was washed with water, then dried over magnesium sulfate, and finally concentrated to give a viscous oil, which without further purification was heated at 100° with 20 cc. of N,N-dimethylaniline for 2 hr. The solution was then partitioned between 5% hydrochloric acid and chloroform, and the chloroform layer was washed with water, then dried over magnesium sulfate, and finally evaporated to yield 810 mg. of oily product. This oily product was hydrogenated in 10 cc. of methanol in the presence of 80 mg. of 10% palladium on carbon. After removal of the catalyst by filtration, the methanolic solution was added to 50 cc. of water and the aqueous solution was extracted with ether. After drying,

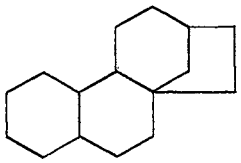
the ether layer was concentrated to give an oily residue which was chromatographed on 100 g. of Merck acid-washed alumina (activity III). The benzene-ether eluent (1:1) gave 325 mg. of 3,3,6-trimethylheptan-4-one, identical in all respects with that obtained from XXIV isolated from Santolina chamaecyparissus L. as described above.

CHAPTER III

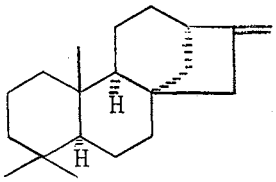
THE PERMANGANATE OXIDATION PRODUCTS OF METHYL FURMAROPIMARATE

Historical and Introduction

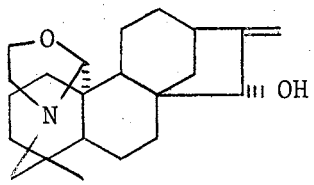
There are a surprisingly large number of naturally-occurring compounds which possess a perhydro-2,10α-ethanophenanthrene skeleton, XXVIII. Examples are the diterpenes of the Phyllocladene group, XXIX, (40-44) and the alkaloids of the Garrya group, XXX (45, 46).



XXVIII

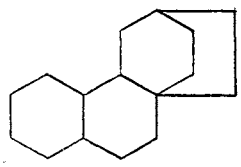


XXIX phyllocladene

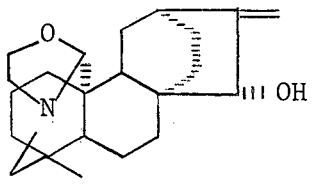


XXX garryfoline

The closely related perhydro-3,10α-ethanophenanthrene skeleton, XXXI, has been observed to a lesser extent in nature, but is found in alkaloids of the Atisine group, XXXII (47, 48).



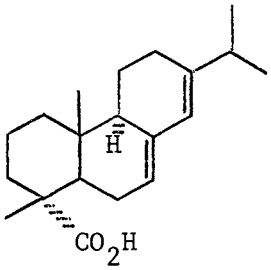
XXXI



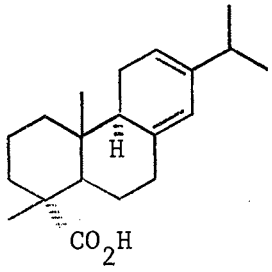
XXXII atisine

Diterpenes possessing carbon skeleton XXXI have not as yet been found in nature, but are to be expected from biogenetic theory. Atisine, XXXII, a member of the Aconite alkaloids, has long been known to be present in the roots of Aconitium heterophyllum, and was first described and named by Broughton (49) in 1877. Jacobs (50) was the first to propose a structure for atisine, but this structure was later corrected by Weisner and his group (51), who assigned structure XXXII (without indicating stereochemistry) to atisine. Much evidence has accumulated in support of structure XXXII and it has been recently reviewed (45-47). Vorbrueggen and Djerassi (52) have established the absolute configuration of atisine and it can now be fully represented by structure XXXII.

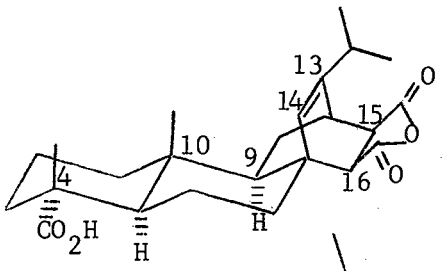
One of the many approaches to the synthesis of diterpenoid alkaloids such as atisine, possessing the bicyclo[2.2.2]octyl C,D ring system has been through the conversion of the resin acid, abietic acid, XXXIII, to the tetracarbocyclic skeletons. Abietic acid was selected because: (a) it is readily available from pine rosin, (b) it is known to undergo Diels-Alder reactions to give bicyclo[2.2.2]octyl C,D ring systems, and (c) it is of known absolute configuration and has been totally synthesized. Starting from the Diels-Alder adducts, the next problem thus becomes one of transforming these adducts into the desired compounds and determining the stereochemistry of the newly formed asymmetric centers. The starting material used by Zalkow and Girotra (53) in their proposed synthesis of compounds containing carbon skeleton XXXI was maleopimaric acid, XXXV, the Diels-Alder adduct of abietic acid and maleic anhydride, and fumaropimaric acid, XXXVI (or XL), the Diels-Alder adduct of abietic and fumaric acids.



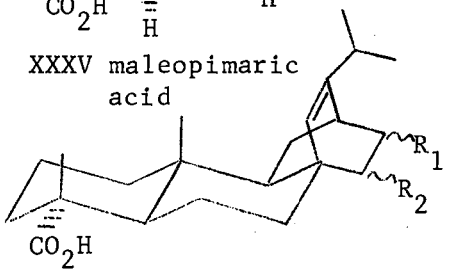
XXXIII abietic acid



XXXIV levopimaric acid



XXXV maleopimaric acid



XXXVI R_1 = CO_2H (dashed)

R_2 = CO_2H (wedge)

XL R_1 = CO_2H (wedge)

R_2 = CO_2H (dashed)

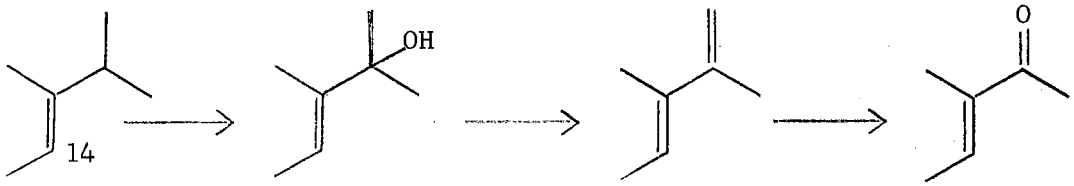
Levopimaric acid, XXXIV, which is present in gum oleoresin, readily condenses at room temperature with maleic anhydride to form the Diels-Alder addition compound, maleopimaric acid, XXXV (54). During distillation of oleoresin, the levopimaric acid present isomerizes to the other resin acids, the end products of the thermal isomerization being abietic acid, XXXIII neoabietic acid, and palustric acid (54). These acids also react with maleic anhydride to give maleopimaric acid but under more vigorous reaction conditions (55). To account for the Diels-Alder reaction in these cases it has been assumed that levopimaric acid is in equilibrium with the other resin acids under conditions required for the reaction (56). Fumaropimaric acid, the trivial name for trans-12,17-dihydrolevopimaric-acid-12,17-endo-α,β-succinic acid, XXXVI (or XL) is the expected product formed in the Diels-Alder reaction of levopimaric and fumaric acids (57).

Maleopimaric acid, XXXV, possesses the carbon skeleton required for synthesis of compounds of structure XXXI, but in addition it also contains several undesirable functional groups. In order to synthesize compounds of carbon skeleton XXXI, the isopropyl and anhydride groups must be removed. Zalkow, Ford, and Kutney (58) investigated the action of alkaline permanganate on maleopimaric acid as a means of determining the stereochemistry of maleopimaric acid and of removing the isopropyl group. However, simultaneous studies by Zalkow and Brannon (see Chapter IV of this thesis) showed that the anhydride moiety in maleopimaric acid was resistant to oxidative bisdecarboxylation. Thus even if the isopropyl group could be removed from maleopimaric acid by permanganate oxidation, there would be no convenient method of removing the anhydride moiety. Zalkow and Brannon did find, however, that methyl fumaropimarate would undergo oxidative bisdecarboxylation to remove the undesired C-15 and C-16 carboxyl groups. Thus a study of the alkaline permanganate oxidation of fumaropimaric acid was initiated in order to remove the undesirable isopropyl group and to determine the stereochemistry of fumaropimaric acid at C-15 and C-16. It is this study with which Chapter III of this thesis is concerned.

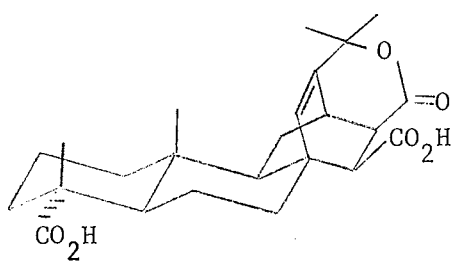
The literature records no past studies of alkaline permanganate oxidation of fumaropimaric acid. Much work has been done, however, on the permanganate oxidation of maleopimaric acid. Due to the similarities in the structure of maleopimaric acid and fumaropimaric acid, it would be reasonable to expect analoguous results from their permanganate oxidation.

The oxidation of maleopimaric acid by alkaline permanganate was first reported by Arbusoz (59), later by Ruzicka and Lalande (60), and

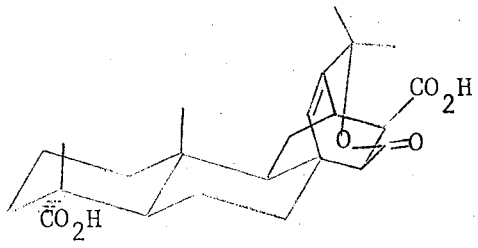
more recently by Zalkow and collaborators (58). Ruzicka and Lalande (60) isolated as the main oxidation product a substance A, $C_{24}H_{32}O_6$. Titration showed A to contain two free carboxyl groups and an unreactive double bond. The two remaining oxygen atoms were assumed to belong to an unreactive lactone group, largely by analogy to the following ozonolysis studies by Ruzicka and Kaufmann (61). Ozonolysis of the trimethyl ester of the acid corresponding to maleopimaric acid, XXXV, led to degradation of the isopropyl side chain to give a diene and an α,β-unsaturated ketone. This was believed to occur via the intermediate alcohol as follows:



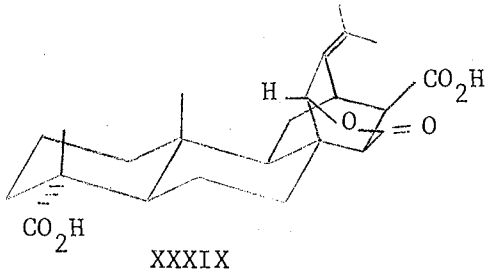
Ruzicka and Kaufmann's (61) ozonolysis results have been recently verified and extended by Zalkow and Girotra (62). Ruzicka (60) isolated three products from the ozonolysis of methyl maleopimarate; the dimethyl ester of one of these products was reported to be identical with the dimethyl ester of substance A (60). On the basis of this evidence structure XXXVII or XXXVIII was suggested for A (60). Zalkow, et al. (58) found neither structure XXXVII nor XXXVIII to be satisfactory for substance A. The infrared absorption spectrum of A showed the presence of a γ-lactones (58). Since both of Ruzicka's structures contain δ-lactones neither could be correct. Through n.m.r. and chemical evidence Zalkow et al. (58) showed substance A to possess structure XXXIX.



XXXVII



XXXVIII



XXXIX

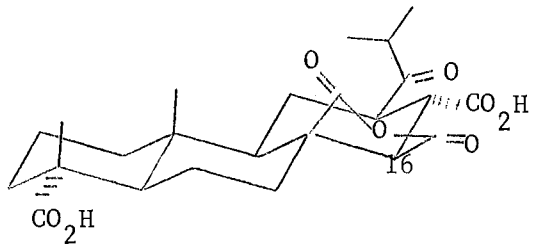
Zalkow's work, when combined with recent reports from other laboratories now allows a complete definition of the absolute configuration of maleopimaric acid and fumaropimaric acid, except for the configuration at C-15 and C-16 in the latter compound. The stereochemistry at C-4, C-5, and C-10, must be the same as in the resin acids. There has been some confusion as to the stereochemistry at C-9 in these adducts since maleopimaric acid has been reported to arise from levopimaric acid and from abietic acid which were reported to differ in stereochemistry at C-9 (63). However, recent work has shown that these two adducts both have an α-hydrogen at C-9 (64). The formation of lactone XXXIX shows that the anhydride moiety of maleopimaric acid is <u>endo</u> to the double bond as would have been predicted on the basis of the known stereochemical selectivity of the Diels-Alder reaction (65). The remaining ambiguity, the stereochemistry at C-8 and C-12, is determined by the approach of the dienophile in the Diels-Alder reaction. Evidence to support the backside approach of the dienophile is now available from several sources. Burgstahler and co-workers (64) have shown that the C ring in levopimaric acid is skewed in such a manner that the β (front) face is shielded by

the angular methyl group at C-10, while the α (rear) face is free for attack by the dienophile. Ayer, McDonald, and Stothers (66) have made a careful study of the position at which the C-10 methyl protons in compounds such as maleopimaric and fumaropimaric acids resonate in the n.m.r. as compared to the C-4 methyl protons and have concluded that the double bond in the Diels-Alder adducts shield the C-10 methyl protons. This observation again requires that the dienophile enter from the α (rear) side, and therefore maleopimaric acid should be pictured sterically as shown in structure XXXV. Based upon the reasoning for the stereochemistry of maleopimaric acid, the absolute configuration of fumaropimaric acid should be represented by structure XXXVI or XL, or a mixture of both.

Results and Discussion

The starting compound, methyl fumaropimarate, XLII, was prepared by heating methyl abietate and fumaric acid together at 200° in a nitrogen atmosphere (67). The dienophile, fumaric acid, would be expected to react with the diene, methyl levopimarate, obtained from thermal rearrangement of methyl abietate, from the less hindered α side. However, two products are still possible -- the C-18 methyl ester of compound XXXVI (XLII) containing an α-C-15 carboxyl group and a β-C-16 carboxyl group, or the C-18 methyl ester of compound XL, containing a β-C-15 carboxyl group and an α-C-16 carboxyl group. The product isolated was shown to be only one of the two possible isomers by its sharp melting point and by the gas chromatograph of its trimethyl ester. The chemical transformations described below show unambiguously that the isomer obtained, XLII, contains an α-C-15 carboxyl group and a β-C-16 carboxyl

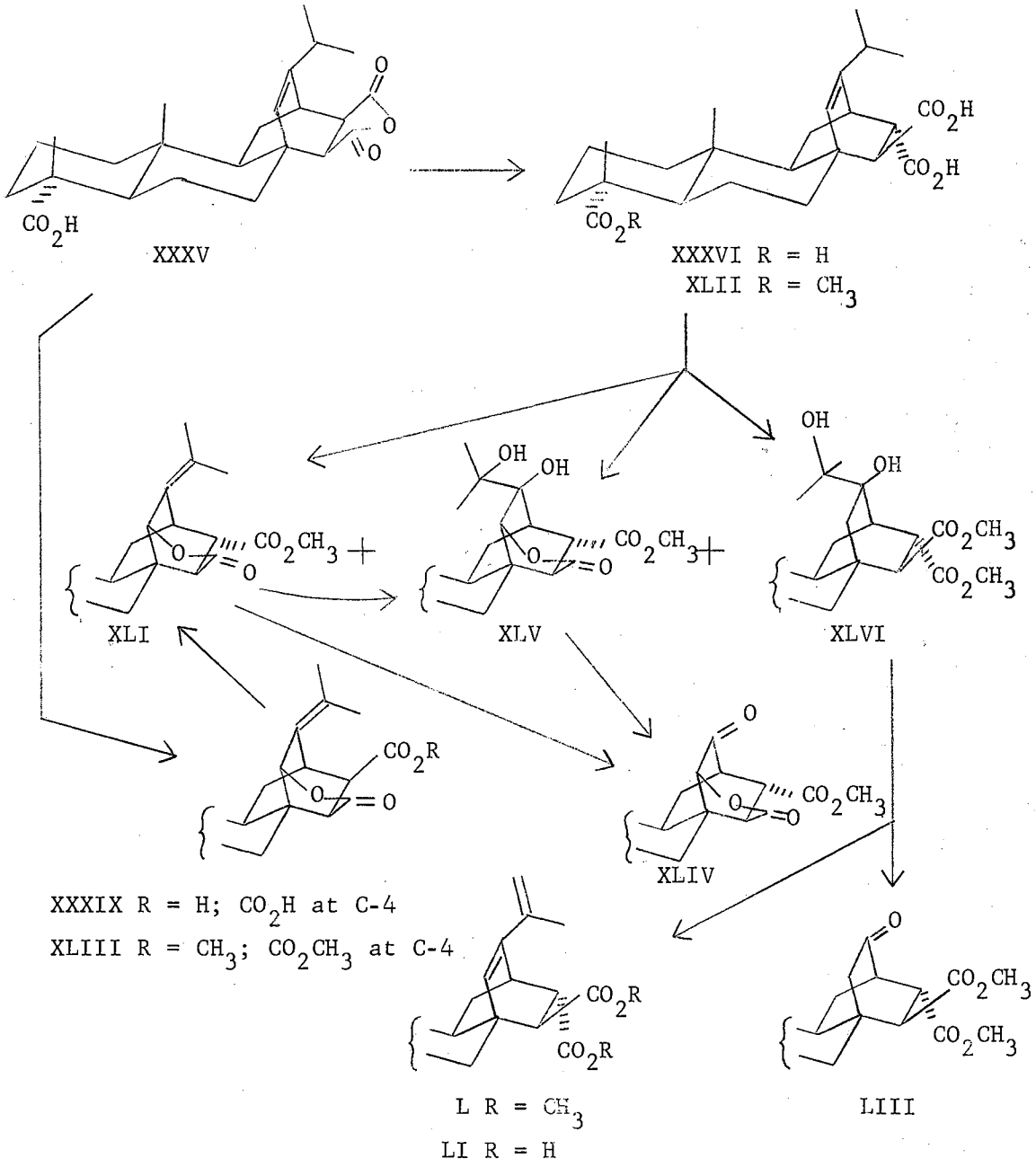
group. An identical isomer was obtained from maleopimaric acid, XXXV, by saponification and epimerization of its trimethyl ester (62). Silver (68) has previously described the epimerization of maleopimaric acid itself, but did not describe the stereochemistry of the epimerization product. Halbrook et al. (69) ozonized fumaropimaric acid and obtained the keto diacid anhydride CVII. From this evidence they reported that fumaropimaric acid had structure XL, containing a β-C-15 carboxyl group and an α-C-16 carboxyl group. However, this conclusion is seemingly contradictory to the requirements for the formation of CVII. The succinic anhydride in CVII could only be formed if fumaropimaric acid contained, not an α-C-16 carboxyl group, but a β-C-16 carboxyl group.



CVII

It was found that fumaropimaric acid and methyl fumaropimarate could be used interchangeably as starting material in the permanganate oxidation with no change in the composition of the crude reaction mixture after esterification with ethereal diazomethane. Oxidation of XLII in alkaline solution with excess permanganate at 10° gave, after acidification and ether extraction, a crude white solid which was esterified with diazomethane then chromatographed on alumina. The infrared spectrum of the first product, XLI, m.p. 177-178°, to be eluted from the chromatography

column showed a strong band indicative of a γ-lactone at 1775 cm^{-1} and an ester carbonyl at 1730 cm^{-1}; its n.m.r. spectrum showed that it was a dimethyl ester which was very similar to XLIII, the latter having been obtained by permanganate oxidation of maleopimaric acid, XXXV, followed by esterification with diazomethane (58).



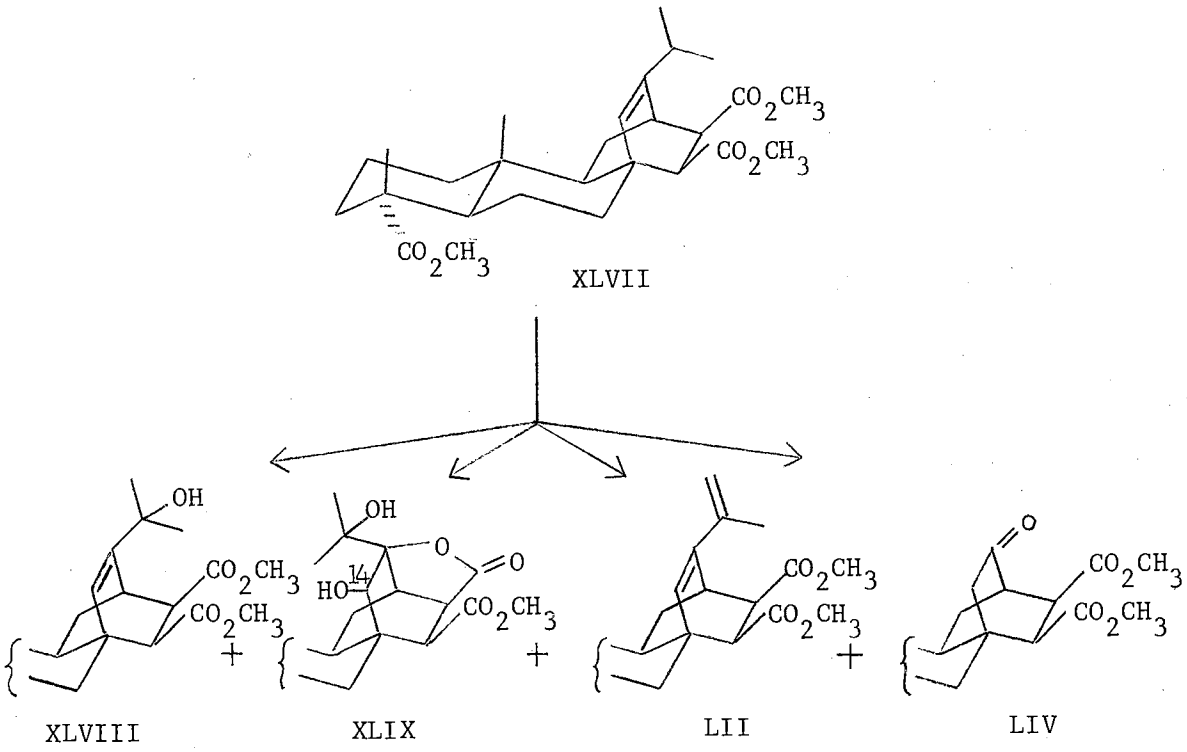
That XLI was the C-15 epimer of XLIII was shown by isomerization of XXXIX with alkali followed by esterification with diazomethane, in which case a mixture of XLIII and XLI was obtained. Further evidence in support of structure XLI was obtained by its ozonolysis to yield ketone XLIV. The infrared spectrum showed a γ-lactone carbonyl band and its n.m.r. spectrum showed that the isopropylidene group was no longer present. It is worthwhile to note that under the same conditions XLIII was unaffected by ozone (58). The lactone XLI has been reported to be formed by the action of lead tetraacetate on XLII, although no experimental details were provided (67). In our hands, the reaction of XLII with lead tetraacetate did not give XLI, but instead two other products were isolated (70).

Establishment of the structure XLI and the alkaline epimerization of the trimethyl ester of maleopimaric acid to XLII and of XLIII to XLI show that the stable isomers have α-C-15 and β-C-16 carboxyl groups rather than the reverse arrangement, and the Diels-Alder adduct obtained from fumaric acid and methyl abietate XLII has this configuration.

Another product (XLV, m.p. 123-125°) isolated by chromatography showed hydroxyl absorption at 3500 cm^{-1}; γ-lactone absorption at 1780 cm^{-1}, and ester carbonyl absorption at 1725-1730 cm^{-1} in its infrared spectrum. The n.m.r. spectrum of XLV showed the C-14 proton at δ 4.50. Oxidation of XLV with lead tetraacetate gave the same ketone XLIV obtained, as mentioned above, from XLI. Thus the structure depicted in formula XLV can be assigned to the product of m.p. 123-125°. Compound XLV undoubtedly arises in the reaction by hydroxylation of intermediate XLI (as free acid) and was indeed readily prepared directly from isolated XLI, after saponification, by further oxidation with permanganate

followed by re-esterification. A comparison of XXXIX and XLI (after saponification) in their reaction with alkaline permanganate is noteworthy. Further oxidation of XXXIX with alkaline permanganate proceeded only with great difficulty and gave a negligible yield of the dihydroxy compound epimeric with XLV at C-15 (58). It can therefore be assumed that hydroxylation of XLI proceeds from the less hindered side cis to the α-carboxy group at C-15 to give a product with the stereochemistry depicted in XLV.

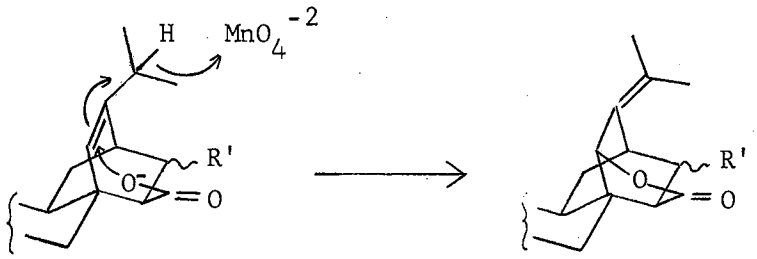
The third and final product (m.p. 206-207^{o}) isolated from the permanganate reaction after esterification and chromatography has been assigned structure XLVI on the basis of the following evidence. The infrared spectrum of XLVI showed -OH absorption at 3500 and 3410 cm^{-1}, and carbonyl absorption at 1715 and 1730 cm^{-1}. Its n.m.r. spectrum showed the presence of three carbomethoxy groups and four methyl group singlets. The two singlets at high field (δ 1.08, 1.13) could be assigned to the C-4 and C-10 methyl groups and the two singlets at next highest field (δ 1.25, 1.32) could be assigned to the two methyl groups flanking the hydroxyl group at C-19 by analogy with XLVIII, one of the products obtained on the treatment of the trimethyl ester of maleopimaric acid, XLVII, with ozone (62).



That the structure of the product of m.p. 206-207° was XLVI and not that arising by normal hydroxylation of the C-13 double bond in XLII was shown by the position of the isopropyl methyl protons in the n.m.r. and by the absence of a signal in the n.m.r. corresponding to the C-14 proton. The latter signal was to be expected at about δ 4.35, the position at which the C-14 proton of XLIX appears (62). Dehydration of XLVI with phosphorus oxychloride in pyridine gave L. The infrared spectrum of L showed no hydroxyl absorption and the ultraviolet spectrum showed a strong band at λmax 245 mμ. The C-15 epimer of L (LII) showed strong absorption at λmax 240 mμ (62). The n.m.r. spectrum of L showed the C-10 methyl protons at δ 0.55, thus providing evidence for the presence of a C-13 double bond (62, 67). The protons of the methyl group

fo the isopropenyl group appeared at δ 1.92, whereas the olefinic protons appeared at δ 5.86 and 6.30, both of these observations are clearly consistent with the assigned structure. Treatment of XLVI with lead tetraacetate gave LIII. The latter compound was identified by comparison with an authentic sample, prepared as previously described (62), by isomerization of LIV. The stereochemistry of the hydroxyl group at C-13 in XLVI is assigned on the same basis as that for XLV as described above.

The formation of XLI and XLV is consistent with a mechanism of the type previously proposed for the formation of XXXIX from XXXV (62).



$$HMnO_4^{-3} + H_2O \longrightarrow MnO_2 + 3OH^-$$

The formation of XLVI is more difficult to rationalize. The C-13, C-14 double bond in XLII does not rearrange to the C-13, C-19 position in the presence of alkali and in the absence of permanganate, but it is possible that the migration does occur in the oxidizing medium.

The above results show the accomplishment of the desired objective of oxidatively removing the isopropyl group of fumaropimaric acid. The absolute configuration of fumaropimaric acid was also determined to be shown in XXXVI, and one of the products, XLVI, has been converted to a

compound, LIII, which by oxidative bisdecarboxylation, after saponification, could lead to synthesis of compounds of carbon skeleton XXXI.

Experimental

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Analyses were performed by Midwest Microlab, Inc., Indianapolis, Indiana. Infrared spectra were recorded using a Beckman IR-5 spectrophotometer. Nuclear magnetic resonance spectra were recorded with the Varian A-60 n.m.r. spectrometer, using tetramethylsilane as an internal standard ($\delta = 0$). Gas chromatographs were run at 280° using a column 0.125 in. by 5 ft. of 5% SE-30 on acid-washed Chromosorb W with a hydrogen flame detector and a hydrogen flow rate of 26 cc. per min. and a nitrogen flow rate of 28 cc. per min. Thin layer chromatograms were run on 25-μ-thick silica gel-coated glass plates using 5:1 benzene-ethyl acetate as the mobile phase, and detection was by iodine vapors.

Preparation of Fumaric Acid-Methyl Abietate Adduct, XLII.

A mixture of 50 g. of commercial methyl abietate (Eastman technical) and 20 g. of fumaric acid was stirred and heated at 200-210° in a nitrogen atmosphere for 3 hrs. After washing the viscous yellow reaction mixture several times with hot water, it was poured into 300 cc. of refluxing benzene. The product XLII crystallized from the cooled benzene solution in 40% yield. The analytical sample was obtained by recrystallization from glacial acetic acid and gave m.p. 294-295° after drying at 144° at 0.1 mm Hg (71). ν_{max}^{KBr} 1730, 1715, and 1960 (weak) cm^{-1}; $[\alpha]_D$ 36° (c 0.004, alcohol).

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42%; H, 8.39%.

Found: C, 69.13%; H, 8.40%.

The adduct XLII was converted into the corresponding trimethyl ester XLVII by treatment with ethereal diazomethane. The triester could not be crystallized but was shown to be homogeneous by gas chromatography, a single peak being obtained in its gas chromatogram with a retention time of 8.7 min. The infrared and n.m.r. spectra of XLVII were identical with those of the product obtained by epimerization and esterification of XXXV (53).

Oxidation of Adduct XLII with Alkaline Permanganate.

The adduct XLII (10 g.) was added to a solution prepared by dissolving 3 g. of sodium hydroxide in 400 cc. of water. After cooling to 10°, a cold 0.165 M aqueous potassium permanganate solution was added to the alkaline solution dropwise until the permanganate color persisted for 3 hours. During the addition of the permanganate solution the reactants were maintained at, or below, 10°. The precipitated manganese dioxide was removed by filtration and gaseous sulfur dioxide was passed through the aqueous solution until the permanganate color disappeared. Acidification (pH 1) with dilute hydrochloric acid gave 5.5 g. of crude crystalline products, and continuous ether extraction (12 hr.) of the aqueous filtrate gave an additional 2.1 g. of products. The combined products were esterified with ethereal diazomethane and separated by column chromatography as described below.

Isolation of XLI.

The crude esterified product (1.58 g.), mentioned above, was chromatographed on 125 g. of Merck acid-washed alumina (activity III) and elution with 15:85 ether-benzene gave 0.320 g. of XLI as a gum which was crystallized from isooctane at -40°. Recrystallization from 1:4 water-methanol and drying at 144° (0.1 mm Hg) gave XLI of m.p. 177-178°,

positive tetranitromethane test, single peak in gas chromatography with retention time of 9.8 min., single spot in thin layer chromatography with R_f 0.625; υ_{max}^{KBr} 1775 and 1730 cm^{-1}; n.m.r. (carbon tetrachloride) δ 0.70 (3 protons), 1.12 (3), 1.78 (6), 3.62 (3), 3.74 (3), and 4.89 (1).

Conversion of XXXIX to XLI.

The lactone XXXIX (90 mg.) prepared as previously described (58) was refluxed in 10 cc. of a 5% sodium hydroxide solution containing 0.5 cc. of methanol for 4 hr. After removal of the solvent with a rotary evaporator the cooled residue was acidified with dilute hydrochloric acid (pH 1) whereupon a white precipitate was obtained. After filtration, the solid product was treated with ethereal diazomethane, and removal of the ether gave a viscous gum which could not be crystallized. An n.m.r. spectrum of this gummy product showed that it was a mixture of XLI and XLIII by the presence of two peaks at high field, δ 0.70 (C-10 methyl protons in XLI) and 0.75 (C-10 methyl protons in XLIII) and by the presence of peaks at δ 3.62 and 3.74. In XLI the two carbomethoxy groups give signals at δ 3.62 and 3.74 whereas in XLIII only one signal (δ 3.62) is observed for the two carbomethoxy groups. The gummy product, after dissolving in 0.5 cc. of methanol, was again refluxed with 10 cc. of a 10% potassium hydroxide solution for 60 hr. The n.m.r. spectrum of the product after re-esterification with diazomethane showed the presence of 40% of XLI. Thin layer chromatography also showed the presence of XLI (R_f 0.625) and XLIII (R_f 0.583).

Isolation of XLVI.

The second product, XLVI, to be eluted in the chromatography of the esterified oxidation product was eluted with 1:1 ether-benzene, whereupon 0.309 g. was obtained from 1.58 g. of the crude esterified product.

This product was crystallized from isooctane and the analytical sample was obtained by recrystallization from methanol, m.p. 206-207° after drying at 100° and 0.1 mm Hg; ν_{max}^{KBr} 3500, 3410, 1715, and 1730 cm^{-1}.

Anal. Calcd. for $C_{27}H_{42}O_8 \cdot CH_3OH$ (71): C, 63.85%; H, 8.80%.

Found: C, 63.92%; H, 8.56%.

Preparation of XLV from XLI.

A solution prepared by dissolving XLI (320 mg.) in 10 cc. of 5% potassium hydroxide and 0.5 cc. of methanol was refluxed for 1 hr., then concentrated to 5 cc. with a rotary evaporator. After cooling to room temperature, a 0.165 M aqueous potassium permanganate solution was added until an excess of permanganate remained. The precipitated manganese dioxide was removed by filtration and gaseous sulfur dioxide was passed through the solution as previously described. The resulting solution was acidified (pH 1) and continuously extracted with ether for 12 hr. The ether extract was dried over magnesium sulfate, concentrated, and finally treated with ethereal diazomethane. Removal of the ether gave 163 mg. of a gummy residue whose n.m.r. spectrum showed very little of the signal at δ 1.78 arising from the isopropylidene protons thus showing that the double bond had reacted. Chromatography of this residue on 55 g. of Merck acid-washed alumina (activity III) gave 80 mg. of pure XLV (m.p. 123-125°) in the 99:1 ether-methanol eluent. The diol XLV thus obtained was identical with XLV (isolated as previously described) in infrared and n.m.r. spectra and the mixture melting point was undepressed.

Ozonolysis of XLI. Preparation of XLIV.

A stream of oxygen-containing ozone (approx. 3%) was passed through a solution, prepared by dissolving 125 mg. of XLI in 15 cc. of glacial

acetic acid, at room temperature for 10 hr. Zinc dust (250 mg.) and water (5 cc.) were then added to the acetic acid solution and stirring was continued for 6 hr. After removal of the zinc by filtration the solution was concentrated with a rotary evaporator and then extracted with four 50 cc. portions of ether. The combined ether extracts were dried over magnesium sulfate and concentrated to give a resinous solid. Chromatography on 40 g. of Merck acid-washed alumina (activity III) gave 90 mg. of ketone XLIV in the 6:4 benzene-ether eluent. The analytical sample was prepared by recrystallization from methanol and gave m.p. 170-172.5°, single spot in thin layer chromatography with R_f 0.580; ν_{max}^{KBr} 1790 and 1730-1740 cm^{-1}; n.m.r. (carbon tetrachloride) δ 0.70 (C-10 methyl protons) and 4.14 (C-14 proton).

Anal. Calcd. for $C_{23}H_{30}O_7$: C, 66.01%; H, 7.23%.

Found: C, 65.70%; H, 7.26%.

Preparation of XLIV from XLV.

Diol XLV (200 mg.) and 275 mg. of dry lead tetraacetate were refluxed in 15 cc. of glacial acetic acid for 24 hr.; an additional 275 mg. of lead tetraacetate was added and refluxing continued for 36 hr. After removal of the acetic acid on a rotary evaporator, 10 cc. of water was added to the residue and the aqueous solution was extracted with three 20 cc. portions of ether. After drying over magnesium sulfate, the ether was removed to give 160 mg. of brown non-crystalline material which was chromatographed on 9 g. of Merck acid-washed alumina (activity III). Elution with 6:4 benzene-ether gave 80 mg. of XLIV, m.p. 168-170°, identical in thin layer chromatography and infrared and n.m.r. spectra with XLIV prepared as described above.

Dehydration of XLVI. Preparation of L.

Diol XLVI (150 mg.) was heated on a steam bath with 2 cc. of phosphorus oxychloride and 5 cc. of dry pyridine for 6 hr. After cooling, 5 cc. of water was cautiously added to the reaction mixture which was then extracted with five 20 cc. portions of ether. The combined ether extract was washed with water, dried over magnesium sulfate, and concentrated to give 110 mg. of non-crystalline residue which was then chromatographed on 10 g. of Merck acid-washed alumina (activity III). Diene L (75 mg.) was eluted in 9:1 benzene-ether as a viscous gum which could not be crystallized. Thin layer chromatography showed one spot with R_f 0.58; υ_{max}^{film} 1730 and 1745 cm^{-1}; λ_{max}^{EtOH} 235 mμ (log ε 4.15). The n.m.r. spectrum (carbon tetrachloride) showed signals at δ 0.55 (3 protons), 1.10 (3), 1.92 (3), 3.60 (3), 3.67 (3), 3.74 (3), 5.86, and 6.30.

Preparation of LIII from XLVI.

Diol XLVI (20 mg.) and dry lead tetraacetate (50 mg.) were refluxed in 15 cc. of glacial acetic acid for 12 hr. After removal of the acetic acid with a rotary evaporator, 20 cc. of water was added and the aqueous solution was extracted with three 25 cc. portions of ether. The combined ether extract was washed with water, then dried over magnesium sulfate. Removal of the ether gave a gummy residue which was chromatographed on 8 g. of acid-washed alumina (activity III). Ketone LIII (12 mg.) was eluted in 9:1 benzene-ether and was crystallized from methanol, m.p. 145°. Thin layer chromatography showed one spot, R_f 0.65; LIII thus obtained was identical in all respects with a sample of LIII prepared as previously described (62).

CHAPTER IV

OXIDATIVE DECARBOXYLATION OF MALEOPIMARIC ACID, FUMAROPIMARIC ACID, AND METHYL FUMAROPIMARATE WITH LEAD TETRAACETATE

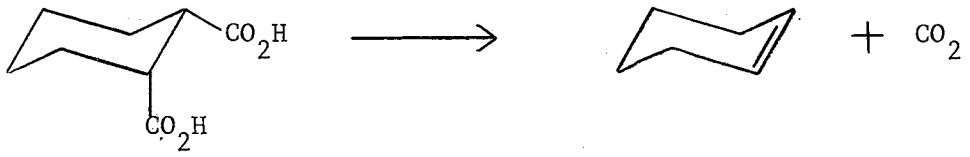
Historical and Introduction

As was elaborated in the Historical and Introduction section of Chapter III of this thesis, maleopimaric acid, XXXV, and fumaropimaric acid, XXXVI, have been studied over the past several years because of their close skeletal relationship to the diterpenoid alkaloids such as atisine, XXXII. One of the important phases of these studies has involved the removal of the D ring carboxyl groups of compounds such as XXXV and XXXVI by use of the oxidative decarboxylation reaction. Early results from such decarboxylation experiments showed the formation of some unusual and unexpected compounds. Thus the oxidative decarboxylation of maleopimaric acid, methyl fumaropimarate, and fumaropimaric acid was studied for the following reasons: (a) to find a suitable means of removing the D ring carboxyl groups in order to lead to compounds of carbon skeleton XXXI, (b) to determine the structures of the unexpected products produced in these oxidation reactions, and (c) in order to understand the mechanisms by which these products arose. Chapter IV of this thesis concerns this study.

Since its first preparation by Dimroth and Schwiezer (72) in 1921, lead tetraacetate has been widely used as an oxidizing agent, the oxidative cleavage of 1,2-diols being one of its more common uses. Only

recently has lead tetraacetate and lead dioxide been used in the oxidative decarboxylation of carboxylic acids to give the corresponding olefins. Although easily prepared, the white, crystalline, tetravalent salt, lead tetraacetate is quickly decomposed by atmospheric moisture to give brown lead dioxide, necessitating its storage in glacial acetic acid, or under high vacuum. In 1934, Oeda (73), treated α-hydroxy acids with lead tetraacetate and obtained the corresponding aldehydes with evolution of carbon dioxide. Although much work had been done with lead tetraacetate and lead dioxide, as late as 1951, no effective method of oxidative decarboxylation was known (74).

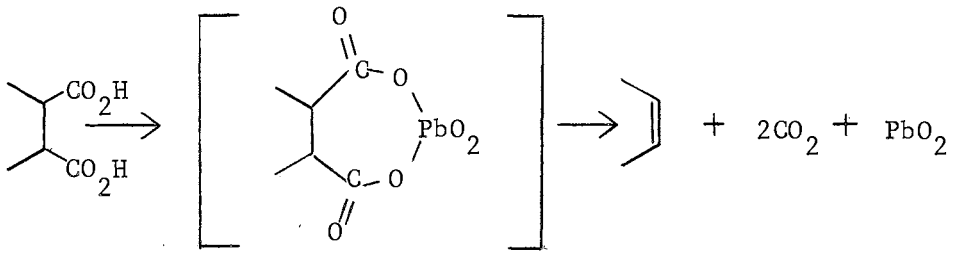
The search for a reagent which would remove two adjacent carboxyl groups in a single operation was intensified by Doering (75), when many of his schemes for the synthesis of 1-substituted bicyclic compounds seemed unfeasible for lack of such a reagent. Doering (75) found two general methods by which the desired reaction could be obtained. By refluxing cis-hexahydrophthalic acid, LV, with lead dioxide in decalin he obtained cyclohexene in 35% yield.



When bicyclo[2.2.2]octane-2,3-dicarboxylic acid, LVI, was heated to 200° with lead dioxide in the presence of ground glass, a 20% yield of bicyclo-[2.2.2]oct-2,3-ene, LVII, was obtained.

LVI LVII

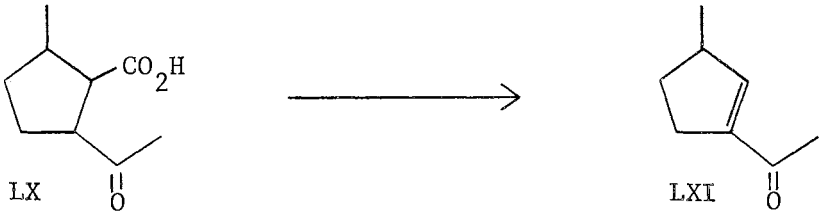
By analogy to the then accepted mechanism of cis diol cleavage by lead tetraacetate, Doering (75) proposed the following as the mechanism of lead dioxide decarboxylation.



Doering realized that decomposition of the reaction intermediate could occur via an ionic or radical process. Two years later, Beckmann and Schaler (76), extended Doering's lead dioxide decarboxylation reaction to include decarboxylation of the anhydrides of dicarboxylic acids. Heating 2-methylbicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride, LVIII, in the presence of a large amount of lead dioxide and ground glass, they prepared 2-methylbicyclo[2.2.1]hept-2-ene, LIX, in good yield.

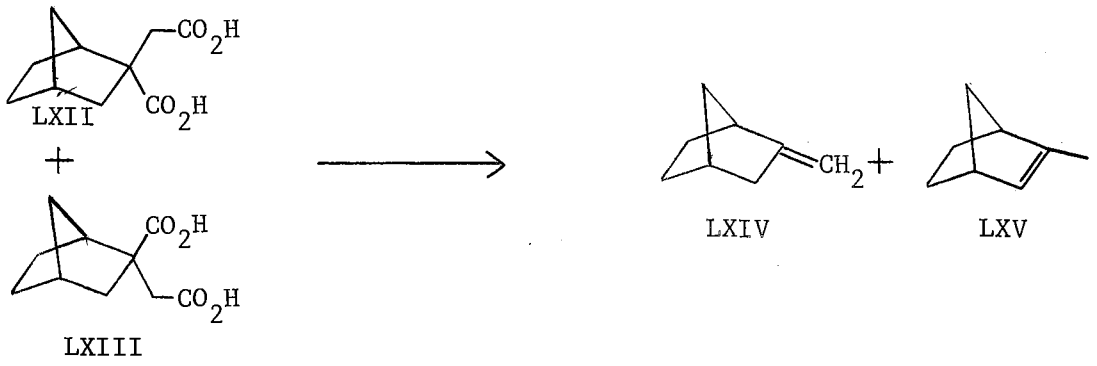
LVIII LIX

They noted that when the reaction was run without the presence of ground glass violent explosions often occurred. Using the same procedure as Doering (75), McElvain and Eisenbraun (77) decarboxylated nepetonic acid, LX, with the elimination of the elements of formic acid and produced the α,β-unsaturated ketone LXI in 34% yield.

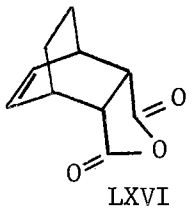


LX LXI

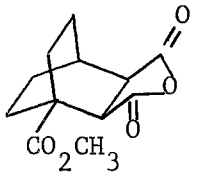
Sauers (78), using Baker's Analyzed lead dioxide, achieved decarboxylation of a mixture of LXII and LXIII and obtained 2-methylene-norbornane, LXIV, and a small amount of LXV.



Other workers (79, 80), however, were not able to obtain decarboxylation with bicyclo[2.2.2]5-octene-2,3-dicarboxylic anhydride, LXVI.



LXVI

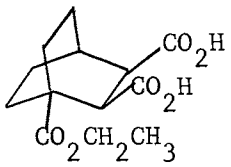


LXVII

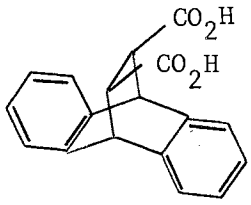
In 1958, both Grob (81) and Doering (82) studied the reproducability of the decarboxylation reaction. Grob and co-workers (81) found that the nature of the lead dioxide used greatly influenced the decarboxylation reaction. Attempts to decarboxylate LXVII gave yields not consistent with the 24% reported by Doering (75). Using freshly prepared commercial samples of lead dioxide, Doering and Finkelstein (82) attempted to repeat Doering's (75) previous decarboxylation of 1-acetobicyclo[2.2.2]octane-2,3-dicarboxylic anhydride. After many attempts only 4% of the theoretical yield of olefin could be obtained. An old sample of lead dioxide of

unknown origin, while not duplicating the original yield, was considerably more active than the fresh material; they also recalled (75) using a single ancient sample of lead dioxide of unknown history. Doering and Finkelstein (82), using emission spectra and x-ray studies, showed that impurities in the lead dioxide were not the cause of the irregularities in the samples of lead dioxide, but effective decarboxylation depended upon the partical size. Attempted decarboxylation of LXVI using samples of lead dioxide, and various samples of Mallinckrodt Analytical Reagent grade all failed. A modification (74) of the hypochlorite oxidation finally led to the production of lead dioxide which would consistently decarboxylate LXVII in 19% yield.

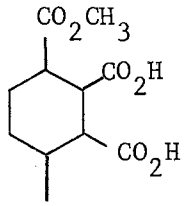
Dissatisfaction with the danger, high temperature requirements (approx. 200°), poor yields, and unpredictability of the lead dioxide procedure of decarboxylation, led Grob (83) to a much more desirable procedure. He showed that when two molar equivalents of base are added to a solution of a molar equivalent of a 1,2-dicarboxylic acid in benzene or acetonitrile and a molar equivalent of lead tetraacetate, decarboxylation occurred below 50° with the formation of olefin and two moles of carbon dioxide gas. Either pyridine or trialkyl amines could be used as the base. By this procedure Grob (83) decarboxylated the cis dicarboxylic acids LXVIII, LXIX, and LXX in 50-70% yield without attack of the lead tetraacetate on the olefin formed in the reaction.



LXVIII



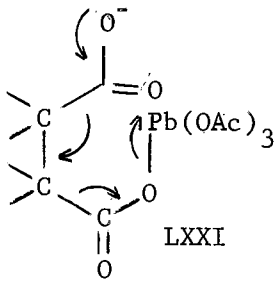
LXIX



LXX

Decarboxylation of trans LXIX could also be effected, however in a lower yield than for the cis isomer. More recently Grovenstein and co-workers (84) have shown that the use of pyridine as the reaction solvent allows a shorter reaction time, lower temperatures, and increased yields compared to the procedure of Grob (83).

Although the oxidative decarboxylation of aliphatic acids by lead tetraacetate has achieved the stature of a standard synthetic tool, many details of the mechanism of this important reaction are obscure. Grob (83) proposed that the reaction mechanism could occur via the formation of intermediate LXXI by a nucleophilic attack of a carboxylate anion on lead tetraacetate, then decomposition of LXXI in a concerted mechanism, or by a direct fragmentation reaction (85).

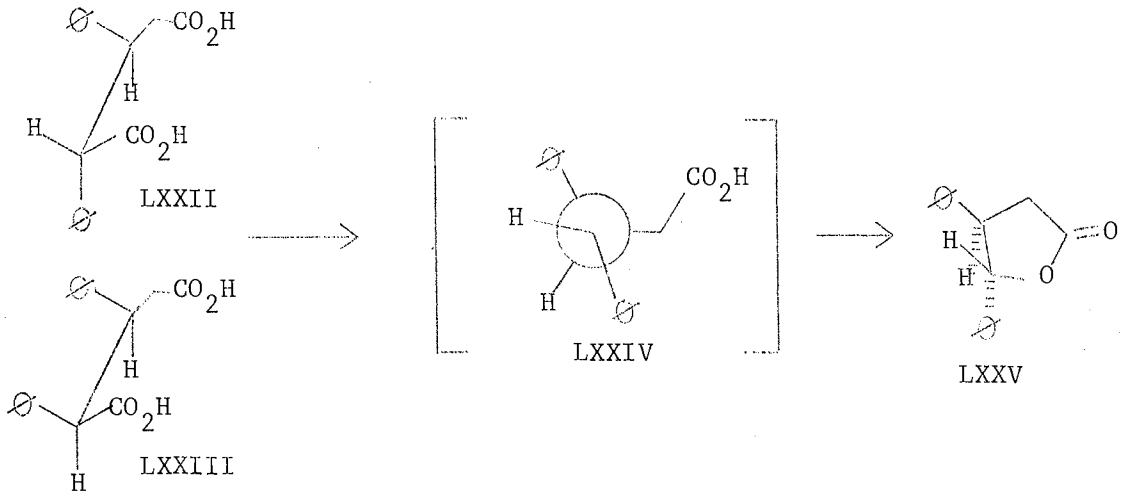


LXXI

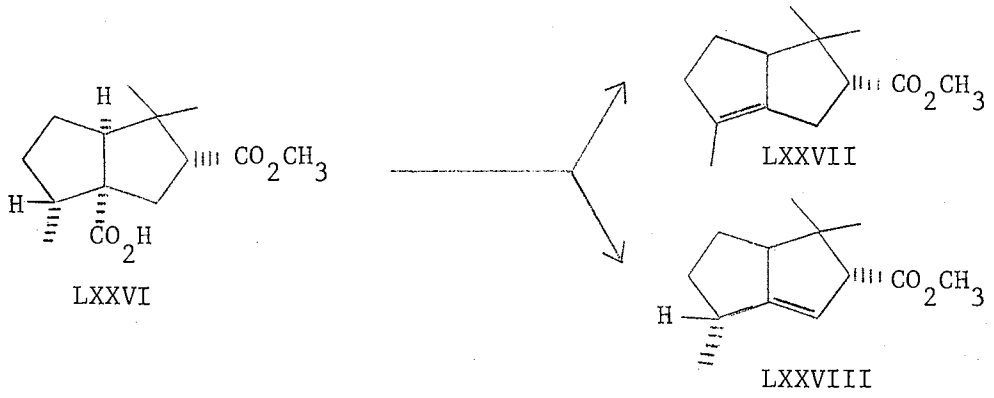
That exchange between organic acids and lead tetraacetate does occur was shown by Mosher and Kehr (86). From a study of the decomposition of organic acids such as formic, acetic, and isobutyric, the following mechanistic scheme was postulated. Interchange first occurs between lead tetraacetate and the organic acid present; decomposition of the Pb^{IV} salt yields the Pb^{II} compound, the negative ion of the acid and a positive ion such as $R\overset{O}{\overset{\|}{C}}-O^{+}$. This electronically deficient oxygen-containing ion may then decompose to carbon dioxide and a carbonium ion which may stabilize itself by losing a proton to form an olefin, by reacting with solvent anion to form esters, or by abstracting a hydride ion from a molecule of solvent

to give rise to a more stable carbonium ion and a hydrocarbon. Mosher and Kehr (86) found no evidence of free radical decomposition of the organic acids studied. On the other hand, the decomposition of lead tetraacetate in acetic acid has been reported by Kharasch and co-workers (87), to involve trivalent lead radicals.

That lead tetraacetate oxidation of organic acids need not be a concerted process was shown by McCoy and Zalago (88) when they obtained the same 3,4-diphenylbutyrolactone from decarboxylation of both isomers, LXXII and LXXIII, of diphenylglutaric acid.



Decarboxylation occurred at the benzylic position to give intermediate LXXIV of sufficient life time to attain the conformation (radical or carbonium ion) which would lead to the most stable lactone, LXXV. Büchi and co-workers (89) proposed much the same mechanism for the decarboxylation of norcedrenedicarboxylic acid monomethyl ester, LXXVI, which gave a mixture of LXXVII and LXXVIII.



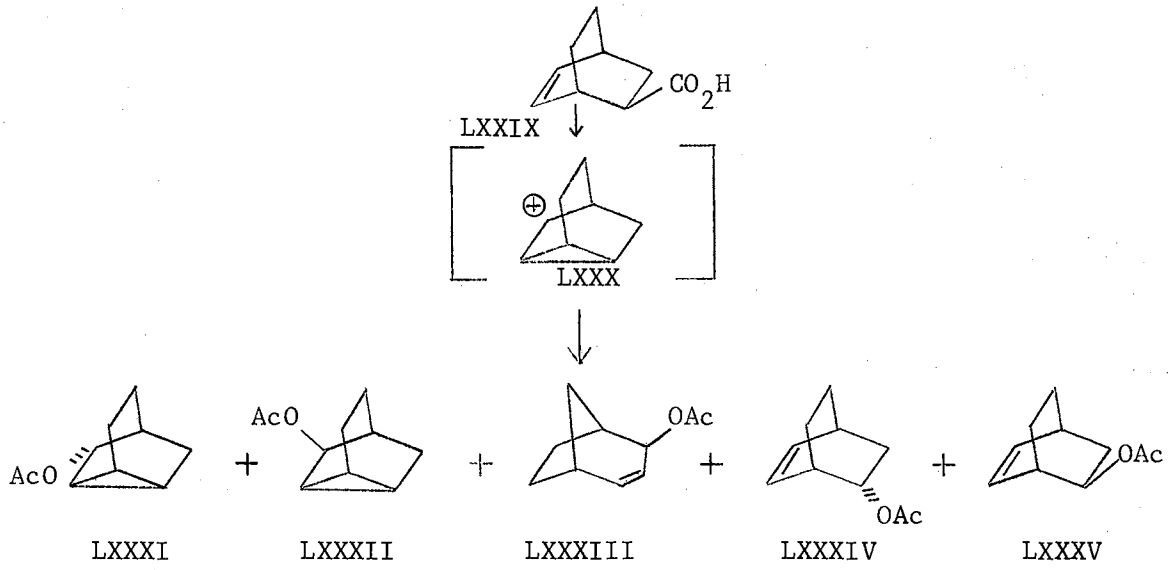
Corey and Casanova (90) found that reaction of lead tetraacetate with either optically active exo- or endo-norbornane-2-carboxylic acid leads to exo-norbornyl acetate as the principal product with the same degree of retention of optical activity in each case. The relatively minor effect of solvent on the stereochemistry of this reaction suggested to Corey and Casanova (90) that the enantiomeric products were formed via a common intermediate, probably a classical norbornyl cation associated with the anion $Pb(OAc)_3^-$ or AcO^-. The norbornyl free radical is definitely excluded as a precursor of rearranged exo-norbornyl acetate on the basis of previous studies which indicate that this intermediate rearranges only at relatively high temperatures. Corey and Casanova, however, could not completely exclude the possibility of a mechanism represented as a homolytic decarboxylation followed by very fast electron transfer from the norbornyl radical to $Pb(OAc)_3$.

That decarboxylation with lead tetraacetate can be forced to proceed through radical or ionic mechanisms has been demonstrated; the former by Barton and Serebryakov (91), and the latter by LaBel and Huber (92). Barton and Serebryakov (91) found that treatment of primary or secondary monocarboxylic acids with lead tetraacetate and iodine in refluxing carbon tetrachloride illuminated with a tungsten lamp gave good yields of the

corresponding nor-iodides. Without addition of the carboxylic acid, lead tetraacetate and iodine reacted as follows:

$$Pb(OAc)_4 + I_2 \longrightarrow 2\ CH_3I + 2\ CO_2 + Pb(OAc)_2.$$

That the reaction proceeds more slowly with lower yield in the dark is consistent with a reaction mechanism of a radical nature. Conversly, the lead tetraacetate decarboxylation (92) of <u>exo</u>-5-carboxybicyclo[2.2.2]oct-2-ene, LXXIX, in glacial acetic acid and potassium acetate afforded a product mixture LXXXI-LXXXV which can best be explained on the basis of initial generation of the classical ion pair LXXX.



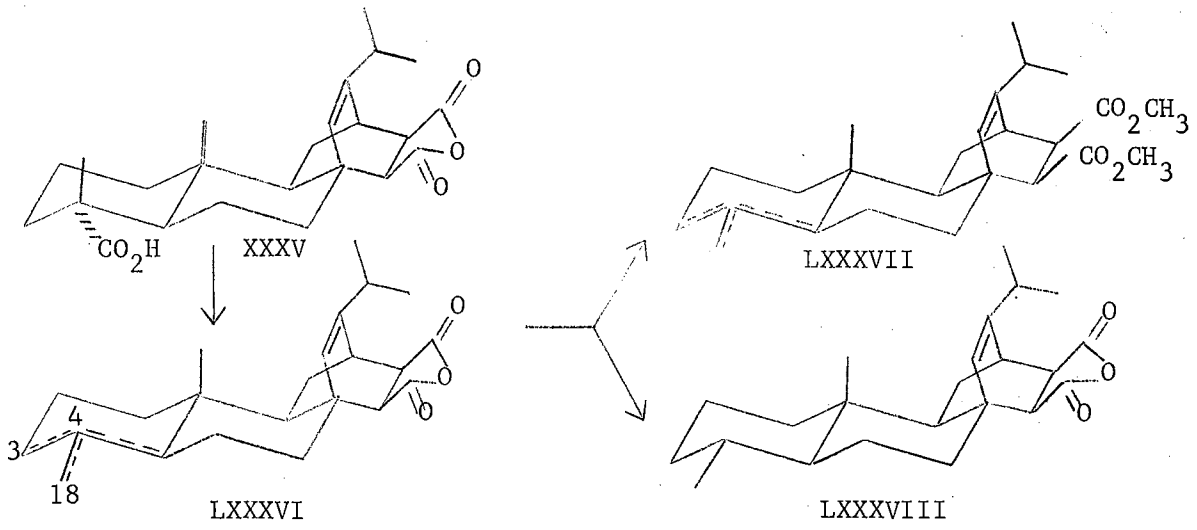
LXXXI LXXXII LXXXIII LXXXIV LXXXV

It has been recently suggested by Starnes (94) that treatment of 3,3,3-triarylpropionic acids with lead tetraacetate does not involve acyloxy radicals, that it does not proceed by competing ionic and radical mechanisms, and that it may involve participation by a neighboring aryl group in the rate-determining decomposition (either homolytic or heterolytic) of the intermediate lead (IV) carboxylate derived from the substrate.

Finally, it is clear that the reactions of lead tetraacetate, including decarboxylation, are connected in a general sense with the problem of of ligand vs. electron transfer mechanism in organic reactions (93). A spectrum of reaction mechanisms is indicated: cationic intermediates are to be expected when these are relatively stable, otherwise free radical or ligand transfer processes should predominate.

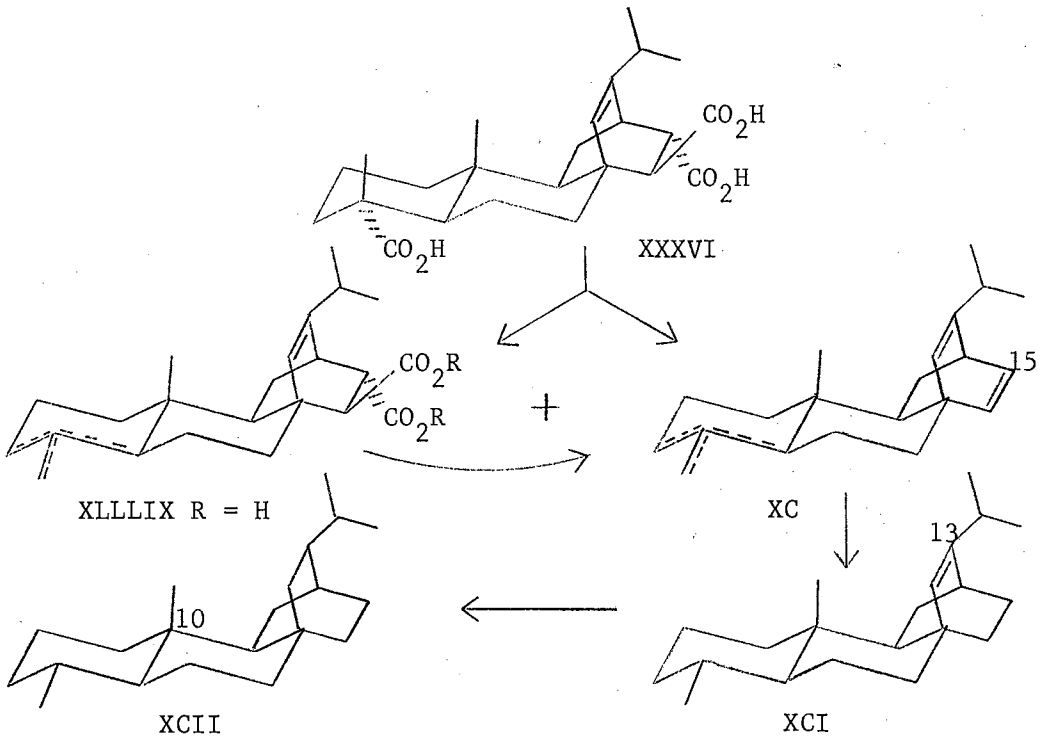
Results and Discussion

Treatment of maleopimaric acid, XXXV, with lead tetraacetate in pyridine (70) at 50° resulted in the vigorous evolution of carbon dioxide with concomitant formation of the diene mixture LXXXVI, in which the n.m.r. spectrum and vapor phase chromatogram indicated the presence of 50% of the $\Delta^{4(18)}$ isomer, 35% of the Δ^{4} isomer, and 15% of the Δ^{3} isomer.



Diene LXXXVI was converted into its dimethyl ester LXXXVII with methanolic diazomethane and readily absorbed a molar equivalent of hydrogen in the presence of platinum oxide catalyst to give LXXXVIII. The double bond at

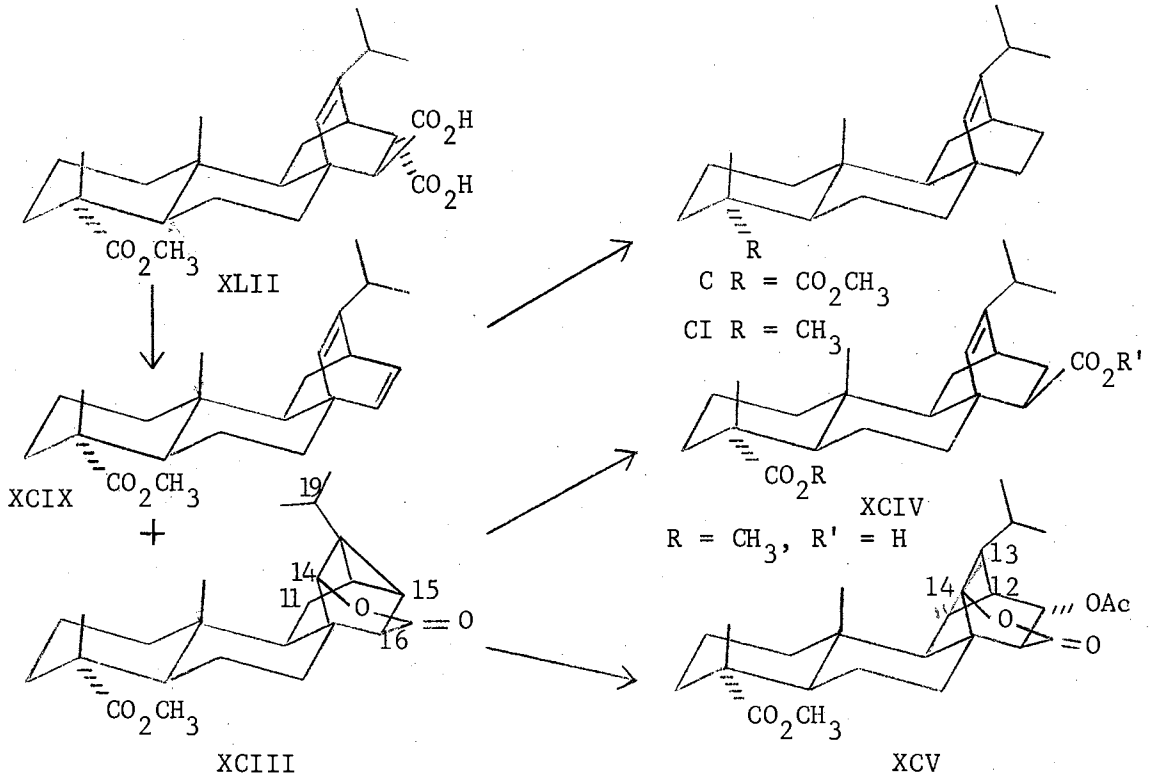
C-13 was not hydrogenated under these conditions as was evident from the n.m.r. spectrum; the methyl group at C-10 appeared at δ 0.54 showing the characteristic shielding by the double bond (62) at C-13, and the C-14 proton appeared at δ 5.48. Under similar conditions fumaropimaric acid, XXXVI, reacted with lead tetraacetate to give LXXXIX as the major product together with some of the triene XC. When LXXXIX was resubmitted to the oxidative decarboxylation reaction, XC was obtained in 25% yield and a lactone later identified as CII was obtained in 65% yield.



Alkaline treatment of LXXXVI also gave LXXXIX. The double bond in the A ring and the double bond at C-15 in XC were readily hydrogenated using platinum oxide catalyst in acetic acid to give the Δ^{13} olefin XCI. The double bond at C-13 in XCI was ultimately reduced to give the saturated hydrocarbon XCII using platinum oxide catalyst in ethanol-acetic

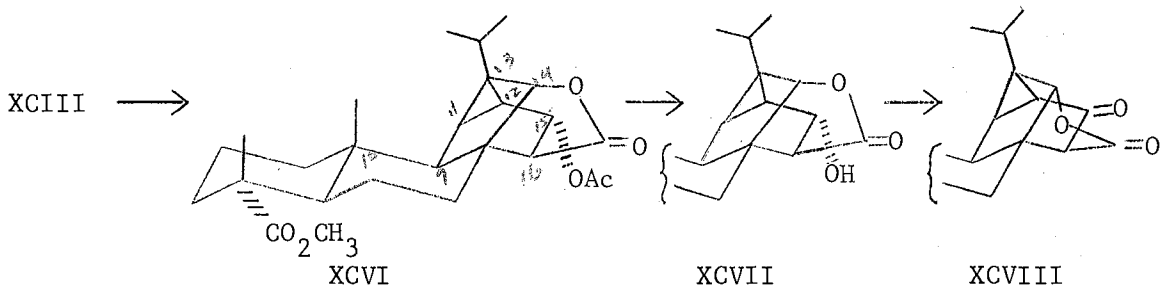
acid and a hydrogen pressure of 40 p.s.i. The n.m.r. spectrum of XCII showed no olefinic protons and the C-10 methyl group gave a signal at δ 0.80.

The reaction of methyl fumaropimarate, XLII, with lead tetraacetate in pyridine gave unexpected results. Chromatography of the crude reaction product on alumina gave as the major product a crystalline compound (m.p. 177-178°) which has been assigned structure XCIII on the basis of the following evidence. The infrared spectrum of XCIII showed the ester carbonyl band at 1730 cm^{-1} and in addition a sharp band appeared at 1775 cm^{-1} which was assigned to a γ-lactone. The n.m.r. spectrum showed a signal at δ 4.5 arising from the proton at C-14 (58) and no olefinic protons appeared to be present; the C-10 methyl group showed no unusual shielding and the isopropyl methyl groups appeared in the region δ 1.10-0.90 showing the absence of a $\Delta^{13(19)}$ double bond.



When the hydrogenation of XCIII was attempted using a large amount of platinum oxide catalyst (35%) one molar equivalent of hydrogen was absorbed and the product isolated was found to be XCIV. The infrared and n.m.r. spectra of XCIV clearly showed that it was an acid-ester; in addition, the n.m.r. spectrum showed the olefinic C-14 proton in the usual place, and the shielding effect of the C-13 double bond on the C-10 methyl group was evident. When an attempt was made to hydrogenate XCIII using a small amount of platinum oxide (4%) in acetic acid, no hydrogen was absorbed but instead a compound B of m.p. 195-196^{o} was obtained. The infrared spectrum of compound B showed the presence of carbonyl bands at 1775 cm^{-1} (γ-lactone) and 1735 cm^{-1} (acetate), whereas its n.m.r. spectrum showed the C-15 acetoxy signal at δ 2.05, the C-14 proton as a singlet at δ 4.75 and the C-15 proton as a doublet ($J_{12,15}$ = 4 c.p.s.) centered at δ 4.94. From this evidence compound B was at first assigned structure XCV, $C_{26}H_{38}O_6$, (70). An inspection of Drieding models indicated that in XCV the dihedral angle between the C-13 and C-14 protons is approximately 90^{o} and a coupling constant of approximately 0 c.p.s. for the C-14 proton would therefore be consistent with structure XCV. Assignment of the acetoxy group at C-15 to an α-configuration is also consistent with the observed coupling of the C-15 proton with the C-12 bridgehead position. However, elemental and mass spectral analysis (see plate III) indicated compound B to have an imperical formula of $C_{26}H_{36}O_6$. Methanolysis of compound B gave an alcohol E, whose analysis showed it to be $C_{24}H_{34}O_5$. Oxidation of alcohol C provided a ketone, D, whose ultraviolet absorption spectrum exhibited a maxima at 203 mμ, indicating a ketone joined to a cyclopropane ring. In the light of this new evidence structure XCV must be discarded

in favor of structure XCVI for compound B (96). Thus compound E has structure XCVII and ketone D has structure XCVIII.



XCVI XCVII XCVIII

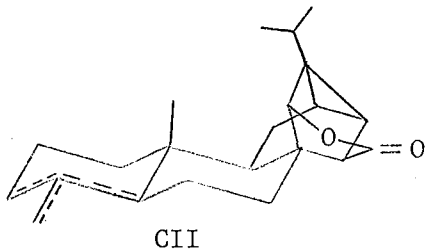
Treatment of XCIII with platinum oxide catalyst in acetic acid without hydrogen still gave XCVI. However stirring XCIII in acetic acid at room temperature without platinum oxide catalyst gave only starting material.

In the oxidation of XLII with lead tetraacetate the expected product XCIX was also produced, but in low yield. As in the previously mentioned cases, the double bond at C-15 was more readily reduced than the double bond at C-13, to give C which was ultimately converted into the hydrocarbon CI. When XLII was subjected to Kolbe electrolysis, XCIII was again produced but no XCIX was detected under these conditions. It has been previously reported (67) that treatment of XLII with lead tetraacetate gave lactone XLI, however, no experimental details of the reaction were reported. The n.m.r. spectrum of the crude decarboxylation product of XXXVI showed no isopropylidene protons, indicating that XLI is not formed by the reaction conditions used in this study (70). Compound XLI is, however, one of the products of alkaline permanganate oxidation of XLII, as discussed in Chapter III of this thesis.

The lactone, previously mentioned, obtained in the oxidative decarboxylation of LXXXIX was assigned structure CII because of the similarity

of its infrared and n.m.r. spectra with those of XCIII.

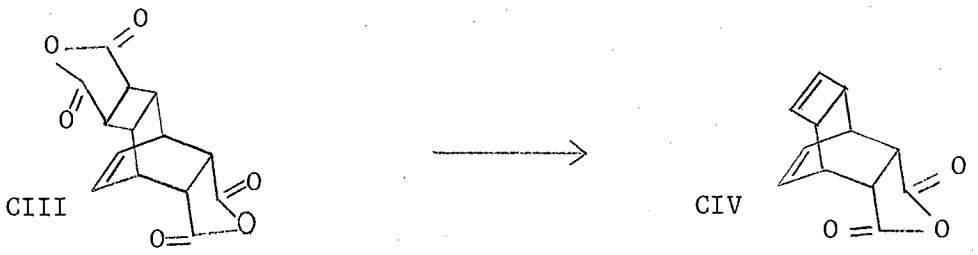
LXXXIX ⟶



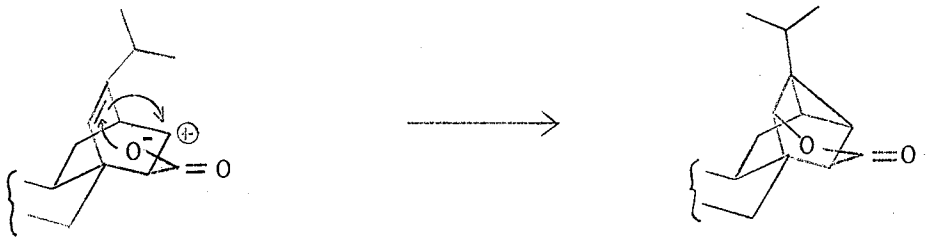
CII

In the reaction of XXXVI with lead tetraacetate the more polar fractions obtained in the chromatography of the crude product showed absorption bands in the infrared at 1775 cm^{-1} presumably due to the presence of CII.

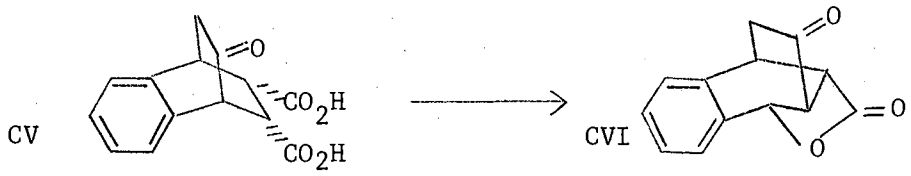
The production of A ring olefins LXXXVI and LXXXIX, as described above, is consistent with the formation of intermediate carbonium ions at C-4. A similar case was recently reported by Büchi et al. (89). The formation of the C-15 double bonds of XC and XCIX provides additional examples of oxidative bisdecarboxylation and a number of related cases can be found in the literature (83). It is clear, however, that in these examples a cyclic intermediate involving the two carboxyl groups cannot be involved. It is interesting that in the reaction of XXXV with lead tetraacetate the anhydride moiety in the D ring was not removed. Grovenstein et al. (84) found that under conditions essentially identical to those used here the <u>endo</u> anhydride group attached to the six-membered ring of CIII was unaffected whereas the anhydride grouping fused to the four-membered ring was lost to yield CIV.



An explanation for these observations may be found in the fact that in both CIII and XXXV the anhydride groups attached to the six-membered rings are not readily opened and thus are unaffected by lead tetraacetate, whereas the anhydride ring fused to the four-membered ring of CIII is highly strained and opens under the oxidative decarboxylation conditions (in the presence of pyridine). Since XXXV is readily converted to XXXVI by alkali, the lack of reactivity of the anhydride moiety in XXXV provides no serious disadvantage if oxidative bisdecarboxylation is desired. The rearrangement required in the formation of XCIII and CII are particularly suggestive of a carbonium ion mechanism and could arise as shown.



Two related cases of oxidative decarboxylation with concomitant lactone formation can be found in the recent literature. Kitahonoki and Takano (95) observed that in the decarboxylation of CV with lead tetraacetate, CVI was isolated as the major product and McCoy and Zagalo (88) found that lead tetraacetate oxidation of 2,3-diphenylglutaric acid gave 3,4-diphenylbutyrolactone, as discussed above.



Both of these reactions are likewise consistent with a carbonium ion mechanism.

In the compounds studied in this investigation the ease of loss of carboxyl groups appears to be C-4 > exo-C-15 > endo-C-16. That the C-4 carboxyl group should be lost most readily is compatible with the known stability of carbonium ions ($3^{o} > 2^{o}$) and loss of the exo-C-15 carboxyl group in preference to the endo-C-16 carboxyl group in LXXXIX and LXII, to give CII and XCIII respectively, may be due to the operation of a concerted mechanism as illustrated above.

The unusual conversion of XCIII to XCVI involves an overall loss of a mole of hydrogen. This can be mechanistically pictured as an acetoxy anion from acetic acid attacking XCIII at C-15 with the loss of a hydride at C-11 resulting in the formation of a cyclopropane ring in XCVI. The hydride then catalytically combines with the proton from the original acetic acid molecule.

Experimental

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Analyses were preformed by Midwest Microlab, Inc., Indianapolis, Indiana. Infrared spectra were recorded with a Beckmann IR-5 spectrometer and n.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, using tetramethylsilane as an internal standard ($\delta = 0$). Ultraviolet spectra were recorded with a Cary Model 14 spectrometer. Gas chromatographs were run at the indicated temperatures using a column

(0.125-in. by 60-in.) of 5% silicone gum rubber (SE-30) on a diatomaceous silica (acid-washed chromosorb W), an Aerograph hydrogen flame detector and nitrogen (28 cc. per min.) as a carrier gas.

Oxidative Decarboxylation of XXXV. Preparation of LXXXVI, LXXXVII, and LXXXVIII.

Maleopimaric acid, XXXV, was dissolved in 75 cc. of dry pyridine, the temperature of the solution raised to 50° and 11.06 g. of dry lead tetraacetate added under a nitrogen atmosphere with stirring. An immediate exothermic reaction ensued with vigorous evolution of carbon dioxide; the color of the solution changed from orange to red and finally to brown. When the evolution of carbon dioxide subsided, an additional 5.53 g. of dry lead tetraacetate was added, but there was no further reaction observed. The entire reaction was complete in less than five minutes. The dark brown solution was cooled, filtered and the filtrate concentrated with a rotary evaporator. After the addition of 300 cc. of a cold 5% hydrochloric acid solution to the gummy residue, the resulting solution was extracted with four 1000 cc. fractions of ether. Concentration of the combined dried either extract gave 7.35 g. of crude non-crystalline product.

Chromatography of 4 g. of the crude product on 200 g. of Merck acid-washed alumina (activity I) gave 3.9 g. of amorphous LXXXVI (m.p. 66-68°) in the ether eluent. Gas chromatography at 280° showed two peaks with retention times of 150 and 158 seconds in a ratio of 18 to 82 respectively. ν_{max}^{KBr} 1845, 1780, 1645, 880, 855 cm^{-1}. The n.m.r. spectrum (in CCl_4) showed the C-10 methyl groups of the mixture at δ 0.45 [0.45 protons Δ^3 isomer], δ 0.54 [1.05 protons $\Delta^{4(5)}$ isomer] and δ 0.75 [1.50 protons $\Delta^{4(18)}$ isomer], the olefinic protons at δ 4.50 (doublet J =

12 c.p.s., 0.50 protons $\Delta^{4(18)}$ isomer] δ 4.72 (0.50 protons $\Delta^{4(18)}$ isomer], and δ 5.28 (0.15 protons $\Delta^{4(5)}$ isomer].

Olefinic mixture LXXXVI (210 mg.), in methanol, was converted into its corresponding dimethyl ester LXXXVII by the addition of an etheral solution of diazomethane. After drying over magnesium sulfate, the solvent was removed and the residue chromatographed on 15 g. of Merck acid-washed alumina (activity I). The dimethyl ester (170 mg.) was obtained from the ether-benzene (2:3) eluent. Two recrystallizations from petroleum ether (b.p. 30-60°) gave the analytical sample, m.p. 122-123°, ν_{max}^{KBr} 1745, 1725, 1645, and 880 cm^{-1}.

Anal. Calcd. for $C_{25}H_{36}O_4$: C, 75.97% H, 9.07; Found: C, 75.96%; H, 9.23%.

A solution of LXXXVI (446 mg.) in acetic acid (15 cc.) in the presence of platinum oxide catalyst (47 mg.) readily absorbed a molar equivalent of hydrogen, then further hydrogen uptake ceased. After removal of the acetic acid with a rotary evaporator, the residue was distributed between a sodium bicarbonate solution and ether. The ether layer was dried over magnesium sulfate and concentrated to give 432 mg. of amorphous LXXXVIII (m.p. 53-55°), ν_{max}^{film} 1845 and 1780 cm^{-1}, n.m.r. δ 0.52 (C-10 methyl), δ 5,42 (C-14 proton).

Oxidative Decarboxylation of XXXVI. Preparation of LXXXIX and XC

Using the same procedure as described above, 10 g. of XXXVI was treated with 12 g. of lead tetraacetate in 100 cc. of dry pyridine to give 7.88 g. of non-crystalline product which was directly esterified with ethereal diazomethane. Gas chromatography at 280° showed that the esterified product contained the three major components LXXXIX (R = CH_3),

XC, and esterified starting material in the ratio of 3.5:1:1.5. Chromatography on 130 g. of Merck acid-washed alumina (activity I) gave 1.6 g. of XC, ν_{max}^{film} 1642, 880, and 705 cm^{-1}; η_D^{25} 1.5360; b.p. 75-77° at 0.1 mm Hg, in the petroleum ether eluent.

Anal. Calcd. for $C_{21}H_{30}$: C, 89.29%, H, 10.71%. Found: C, 89.52%, H, 10.33%. The n.m.r. spectrum of XC was similar to that of LXXXVI, but, in addition, it showed the C-15 and C-16 protons as the AB part of an ABX system in the region δ 5.84-6.34.

The ether-benzene (1:4) eluent from the chromatography of the esterified crude product yielded 4.8 g. of LXXXIX (R = CH_3), b.p. 153-155° at 0.1 mm Hg, ν_{max}^{film} 1730, 1645, and 880 cm^{-1}. The n.m.r. spectrum of LXXXIX (R = CH_3) was similar to that of LXXXVI. Further elution (ether-benzene 1:1) gave 1.1 g. of unreacted esterified starting material and finally elution with ether gave material whose infrared spectrum showed absorption at 1775 cm^{-1} (γ-lactone).

The diester LXXXIX (R = CH_3) was also obtained from LXXXVI as follows. Anhydride LXXXVI (200 mg.) was refluxed in a solution of 0.5 cc. of methanol and 5 cc. of 10% aqueous potassium hydroxide for 9 hrs. After cooling, the solution was acidified with dilute hydrochloric acid and the resulting precipitate was esterified by the addition of excess ethereal diazomethane. The crude esterified product was chromatographed on 9 g. of Merck acid-washed alumina (activity III) whereupon 90 mg. of LXXXIX (R = CH_3) was obtained from the benzene eluent. The diester so obtained was identical in all respects with that obtained as described above.

Oxidative Decarboxylation of LXXXIX (R = CH_3). Preparation of XC and CII

Diester LXXXIX (R = CH_3) was hydrolyzed by refluxing in 10% aqueous

potassium hydroxide for three hours. Acidification of the solution with dilute hydrochloric acid and filtration of the precipatate gave the di-acid LXXXIX, (R = H) m.p. 140-143°, ν_{max}^{KBr} 2850-3100, 1715, 1645 and 880 cm^{-1}.

Diacid LXXXIX (2.50 g.) was decarboxylated as previously described to give 1.9 g. of crude viscous product to which ether was added to give a 65% yield of lactone CII. Recrystallization from acetic acid gave m.p. 310-313°, ν_{max}^{KBr} 1775 cm^{-1}.

Anal. Calcd. for $C_{22}H_{30}O_2 : CH_3CO_2H$: C, 74.61; H, 8.85%.

Found: C, 74.36; H, 8.37%.

Chromatography of the remaining crude product, after the removal of CII, on Merck acid-washed alumina (activity III) gave a 25% yield of XC in the petroleum ether eluent. The XC thus obtained was identical in infrared and n.m.r. spectra with XC obtained as previously mentioned.

Hydrogenation of XC. Preparation of XCI and XCII

When 300 mg. of XC in 20 cc. of acetic acid was hydrogenated at room temperature and atmospheric pressure in the presence of 38 mg. of platinum oxide, 2 molar equivalents of hydrogen were absorbed within 2.5 hr., after which all hydrogen uptake ceased. After removal of the catalyst by filtration, 100 cc. of aqueous sodium bicarbonate solution was added to the filtrate which was then extracted with ether. Evaporation of the ether extract, after drying over magnesium sulfate, gave 254 mg. of XCI, b.p. 65-70° at 0.1 mm Hg., η_D^{25} 1.5208, ν_{max}^{film} 2950 and 1640 cm^{-1}, n.m.r. (CCl_4): δ 5.45 (C-4 proton). When XCI was further hydrogenated in acetic acid-ethanol at 40 p.s.i.g. and room temperature in the presence of platinum oxide for 12 hrs., one molar equivalent of hydrogen was absorbed to give XCII, b.p. 70-72° at 0.1 mm Hg, η_D^{25} 1.5165, ν_{max}^{film} 1640 and 1380 cm^{-1}.

Oxidative Decarboxylation of XLII. Preparation of XCIII and XCIX.

XLII (10 gm.) was added to 80 cc. of dry pyridine in a nitrogen atmosphere, the temperature of the solution raised to 50° and 14.23 g. of dry lead tetraacetate was added in one portion. Within three minutes carbon dioxide evolution ceased and the solution had become black in color. The solution was filtered and the filtrate concentrated with a rotary evaporator. Dilute hydrochloric acid (500 cc. of 5%) was added to the concentrate and the aqueous solution was exhaustively extracted with ether. After drying over magnesium sulfate, the ether extract was evaporated to yield 7.85 g. of crude non-crystalline product.

Chromatography of the crude product on 500 g. of Merck acid-washed alumina (activity III) gave 1.22 g. of XCIX in the ether-benzene (1:9) eluent as a viscous oil, b.p. 140° at 0.1 mm Hg, η_D^{25} 1.5163, ν_{max}^{film} 1730, 1645 and 705 cm^{-1}, which showed a single peak at 230° with a retention time of 115 sec. in its gas chromatogram. The n.m.r. spectrum of XCIX in carbon tetrachloride showed the C-14 proton as a singlet at δ 5.42 and the C-15 and C-16 protons appeared as the AB portion of an ABX system in the region δ 5.94-6.28. (See analysis of dihydro derivative below.) Further elution with ether-benzene (1:1) gave 4.3 g. of lactone XCIII which after two recrystallizations from acetic acid gave m.p. 177-178°, ν_{max}^{KBr} 1775 and 1730 cm^{-1}.

Anal. Calcd. for $C_{24}H_{34}O_4$: C, 74.57%; H, 8.86%.

Found: C, 74.27%; H, 8.75%.

A sample of the crude reaction product was treated with excess ethereal diazomethane. The n.m.r. spectrum of the esterified product showed no isopropylidine protons indicated the absence of any lactone XLI.

Hydrogenation of XCIX. Preparation of C.

When diene XCIX (116 mg.) was hydrogenated in acetic acid (15 cc.) using platinum oxide catalyst (26 mg.) at atmospheric pressure and room temperature, one molar equivalent of hydrogen was rapidly absorbed. After the usual workup, 110 mg. of the olefin C was obtained, b. p. 140° at 0.1 mm Hg, η_D^{25} 1.5163, ν_{max}^{KBr} 1740, 1645, and 1245 cm^{-1}, n.m.r. (CCl_4) δ 0.58 (C-10 methyl), δ 5.39 (C-14 proton).

Anal. Calcd. for $C_{23}H_{36}O_2$: C, 80.18%; H, 10.53%.

Found: C, 80.03%; H, 10.58%.

Conversion of C to CI.

C (5.86 g.) in 30 cc. of anhydrous ether was added, with stirring, to a solution containing 0.68 g. of lithium aluminum hydride in 100 cc. of ether and the entire solution was refluxed for 3 hrs. After the usual workup, 4.54 g. of the corresponding alcohol (ν_{max}^{film} 3410 cm^{-1}) was obtained, and was transformed into its p-toluenesulfonate by refluxing with p-toluenesolfonyl chloride in pyridine. The tosylate (3.34 g.) was directly reduced by refluxing with lithium aluminum hydride (0.42 g.) in dioxane (50 cc.) for 17 hrs. to give 2.15 g. of colorless liquid product which was chromatographed on 120 g. of Merck acid-washed alumina (activity I). The petroleum ether eluent gave CI, b.p. 70° at 0.1 mm Hg, η_D^{25} 1.5133, ν_{max}^{film} 2925 and 1460 cm^{-1}.

Anal. Calcd. for $C_{22}H_{36}$: C, 87.89%; H, 12.11%.

Found: C, 87.63%; H, 17.22%.

The ether-benzene (1:1) eluent gave 1.51 g. of the starting alcohol. The yield of CI was greatly improved by using the following procedure (53). The alcohol (1 g.) obtained by reduction of C with lithium aluminum hydride, in 10 cc. of pyridine was added to a solution of 0.5 g.

of chromic anhydride in 10 cc. of pyridine and after stirring at room temperature for 3 hrs. the solution was poured into 100 cc. of ice water. The aqueous solution was extracted with ether and after washing and drying the ether extract was evaporated to give 0.88 g. of crude aldehyde (ν_{max}^{film} 2680 and 1725 cm^{-1}) which was directly reduced as follows. The aldehyde (0.88 g.) was refluxed with 1.5 g. of potassium hydroxide, 2 cc. of hydrazine, and 10 cc. of diethylene glycol for 3 hrs., then the solution was distilled, removing water and hydrazine until the temperature reached 240°, when 1.5 cc. of hydrazine was again added and refluxing continued for an additional 10 hrs. The solution was then poured into water and the aqueous solution extracted with ether. After washing with water, the ether extract was dried over magnesium sulfate and evaporated to give 720 mg. of crude product which gave 520 mg. of CI after chromatography on alumina.

Preparation of XCIV.

A solution prepared by dissolving 0.39 g. of XCIII in 10 cc. of glacial acetic acid was stirred in the presence of 0.14 g. of platinum oxide in a hydrogen atmosphere; over a period of 2 hrs., 22.1 cc. of hydrogen was absorbed. The solution was worked up as described above to give 0.37 g. of crude product XCIV, ν_{max}^{film} 2800-3100, 1740, 1715 and 1645 cm^{-1}, n.m.r. (CCl_4): δ 0.60 (C-10 methyl), δ 3.60 (methyl ester in the A ring), δ 5.34 (C-14 proton), δ 10.15 (carboxyl proton). XCIV was methylated with ethereal diazomethane and the product chromatographed on alumina to give, in the ether-benzene eluent (1:4), XCIV (R = R' = CH_3) as a viscous oil, ν_{max}^{film} 1740, 1735, and 1645 cm^{-1}, n.m.r. spectrum similar to that of XCIV. XCIV (R = R' = CH_3) (250 mg.) was saponified by refluxing for 6 hrs. with 2.5 cc. of 3N aqueous sodium

hydroxide and 1.0 cc. of methanol. The diacid XCIV (R = R' = H), obtained by acidification of the above solution, was twice recrystallized from carbon disulfide-ether (7:3) to give m.p. 194-196°, ν_{max}^{KBr} 3100-2800, 1710-1715, and 1645 cm^{-1}.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.75%; H, 9.15%.

Found: C, 73.29%; H, 9.25%.

Preparation of XCVI.

A solution containing 3 g. of XCIII and 0.12 g. of platinum oxide in 20 cc. of glacial acetic acid was stirred in a hydrogen atmosphere at room temperature for 36 hrs., but no hydrogen uptake was observed. The catalyst was removed by filtration and the acetic acid solution was then poured into 200 cc. of water. The aqueous solution was exhaustively extracted with ether, and the ether extract was washed with water then dried over magnesium sulfate. Evaporation of the ether layer gave 2.83 g. of crude XCVI, m.p. 195-196°, ν_{max}^{KBr} 1775, 1735 and 1300 cm^{-1}. The mass spectrum of XCVI (CEC Model 21-103C) exhibited only a very small molecular ion peak (m/e 444, 0.38%). However, a larger peak (6.66%) was found at m/e 384 (m-60) which undoubtedly arises by loss of acetic acid from the molecular ion and a still larger peak (36.34%) was observed at m/e 340 (m-60-44) which would correspond to loss of acetic acid and the isopropyl group as propane. The corresponding peaks to be expected of XCV (m 446) were either absent or negligible in size. (see Plate III). N.m.r. (CCl_4) doublet centered at 0.86 (J = 7 c.p.s., 3 protons) δ 0.95 (3 protons), doublet centered at δ 1.12 (J = 7 c.p.s., 3 protons), δ 1.20 (3 protons), δ 1.92 (1 proton), δ 3.65 (3 protons), δ 4.68 (1 proton), doublet centered at δ 4.97 (J = 5 c.p.s., 1 proton).

Anal. Calcd. for $C_{26}H_{36}O_6$: C, 70.25%; H, 8.16%.

Found: C, 70.52%; H, 8.14%.

XCVI was also obtained in 55% yield when the reaction was repeated under identical conditions except in a non-hydrogen atmosphere. Stirring XCIII in glacial acetic acid at room temperature for 40 hrs. in a non-hydrogen atmosphere, however, resulted in quantitative recovery of XCIII upon removal of the acetic acid by vacuum.

Preparation of Alcohol XCVII.

100 mg. of XCVI in 10 cc. of anhydrous methanol and 0.1 g. of sodium methoxide was stirred at room temperature for 7 hrs. After removal of the methanol at room temperature under vacuum the viscous residue was poured into 100 cc. of water and extracted with three 25 cc. portions of ether. The ether extracts were dried over magnesium sulfate, the ether removed, and the resulting residue chromatographed on 50 g. of Merck acid-washed alumina (activity III). Benzene-ether (3:10) gave 84 mg. of crystalline hydroxy-lactone XCVII. Recrystallization from benzene-pet ether (1:4) gave an analytical sample, m.p. 190-191°, ν_{max}^{KBr} 3500, 1780, 1730 cm^{-1}, n.m.r.

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61%; H, 8.52%.

Found: C, 71.98%; H, 8.47%.

Oxidation of XCVII. Preparation of XCVIII.

0.5 cc. of Jones Reagents (26.72 g. of chromic anhydride, 28 cc. of conc. sulfuric acid, and 72 cc. of water) was added dropwise to a stirred solution of 20 mg. of XCVII in 3 cc. of acetic acid at room temperature. After 30 min. the acetic acid was removed at room temperature by vacuum and 10 cc. water added to the residue. The resulting aqueous suspension was extracted with three-15 cc. portions of ether. After washing with

water and drying over magnesium sulfate, the ether extracts condensed to give a viscous gum which crystallized upon addition of heptane to give crystals which melted 174-176°. $\lambda_{max}^{spectro.\ CH_3OH}$ 203 mμ (ε = 2100). υ_{max}^{KBr} 1785, 1715-1725 cm^{-1}.

Anal. Calcd. for $C_{24}H_{32}O_5$: C, 71.96%; H, 8.07%.

Found: C, 71.67%, H, 8.09%.

Preparation of XCIII by Kolbe Electrolysis.

Diacid XLII (5.0 g.) in 200 cc. of 5% aqueous sodium hydroxide and 60 cc. of methanol was electrolyzed for 3 hrs. using a current of 2 amps. and 22 volts D.C. at 23° in a cell equipped with platinum electrodes and a commutater. At the end of this period, 700 cc. of water was added and the aqueous solution was extracted with ether. The dried ether extract yielded 0.33 g. of neutral material. The alkaline aqueous solution was then acidified with cold hydrochloric acid and the precipitate formed was filtered and dried, 3.7 g., m.p. 290-293°. The infrared spectrum and mixed melting point showed this material to be identical to starting diacid XLII. The neutral fraction (300 mg.) was chromatographed on 10 g. of Merck acid-washed alumina (activity III) and the ether-benzene (1:1) eluent yielded 205 mg. of XCIII, identical in all respects with that obtained in the reaction of XLII with lead tetraacetate.

Plate I

Mass Spectrum of 3,3,6-Trimethyl-1,5-heptadiene-4-one (XXIV)

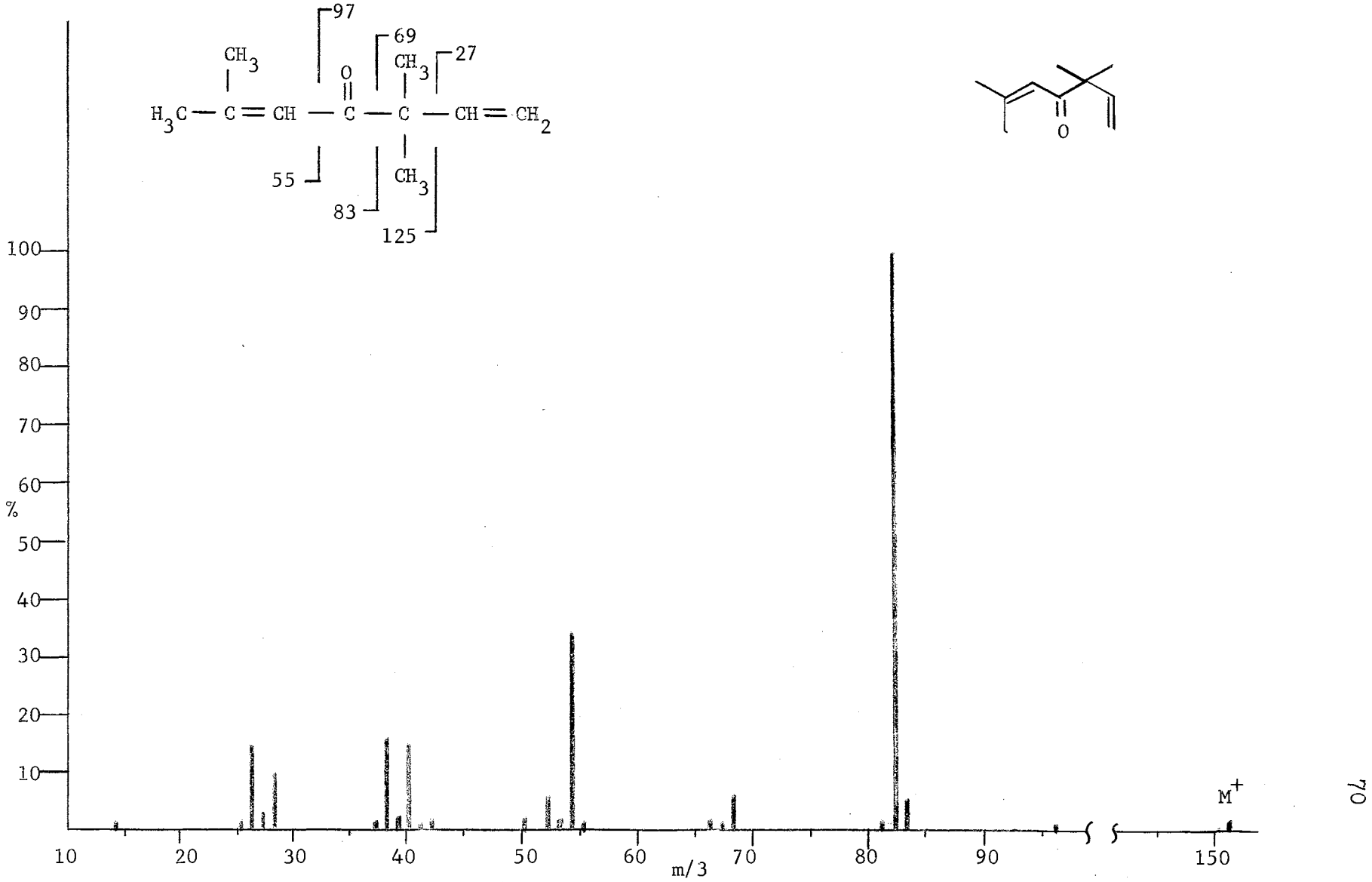


Plate II

Mass Spectrum of 3,3,6-Trimethylheptan-4-one (XXV)

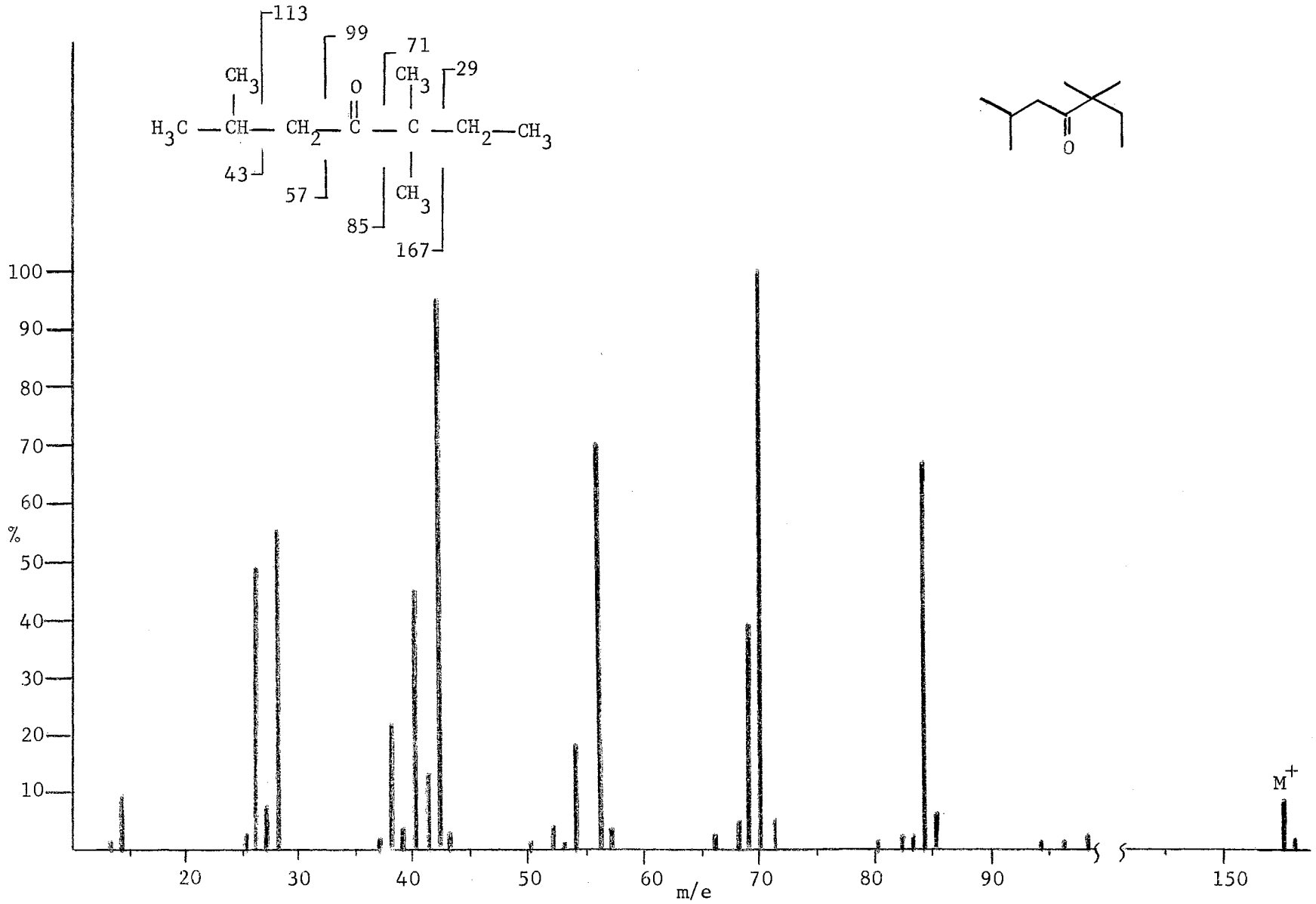
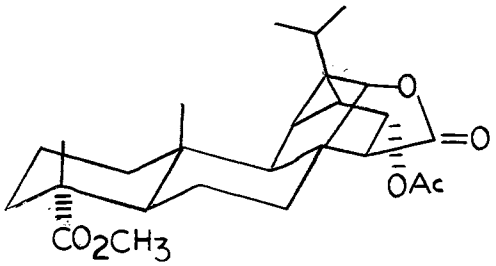


Plate III

Mass Spectrum of Lactone XCVI



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