

ISOLATION AND FRACTIONATION OF TREMETOL
FROM RAYLESS GOLDENROD

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ISOLATION AND FRACTIONATION OF TREMETOL
FROM RAYLESS GOLDENROD

By
CLARENCE A. LATHROP
Bachelor of Science
New Mexico State College
State College, New Mexico
1937

Submitted to the Department of Chemistry
Oklahoma Agricultural and Mechanical College
In Partial Fulfillment of the Requirements
For the degree of
MASTER OF SCIENCE
1939

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APPROVED:

O. C. Derner

In Charge of Thesis

Otto M. Smith

Head of the Department of Chemistry

D. C. Mitchell

Dean of the Graduate School

118299

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. O. C. Dermer under whose direction and with whose help this work has been done. He also wishes to acknowledge the assistance rendered by Dr. J. F. Couch by the suggestions he has made and by the sample of 'tremetol' he sent for comparison. Dr. Paul Arthur, Dr. C. L. Nickolls and Mr. Ernest Utterback have also contributed valuable aid for which the author is sincerely grateful.

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INTRODUCTION

Rayless goldenrod (Aplopappus heterophyllus, Blake) is found in the Southwestern section of the United States, and in particular abundance in the Pecos Valley of New Mexico. Investigators have found the ingestion of the plant to be responsible for the disease variously known as milksickness, trembles and 'Alkali' disease.

Scientific investigation of rayless goldenrod has dealt largely with (1) feeding experiments to prove the toxicity of the plant to animals, and (2) the separation of a toxic fraction from the plant by Couch of the United States Department of Agriculture. His work is the only previous chemical investigation made of the poison. He assigned the name tremetol and the empirical formula of $C_{13}H_{22}O_8$ to the toxic fraction which he isolated.

The author became interested in the poisonous properties of rayless goldenrod owing to the efforts of his father, a veterinary in the Pecos Valley of New Mexico, to combat the great loss of live stock in those parts. It has been the author's thought that if a structural formula could be assigned to the poison, an antidote might be found whereby affected animals could be cured. At present there is no specific nor very successful cure for goldenrod poisoning.

It was with this view that the present chemical investigation was started, in the hope of contributing to the establishment of a structural formula for the poison in rayless goldenrod.

HISTORICAL

Rayless goldenrod (Aplopappus heterophyllus, Blake; also called Isocoma heterophylla) which occurs in the Southwestern section of the United States, was proved to be toxic to animals by experiments carried out by Marsh, Roe and Clawson (5,6) in 1921 at Roswell, New Mexico. These investigators found that the feeding of rayless goldenrod produced symptoms identical with those obtained by the ingestion of richweed or white snake root, found in the mid-western states. Both of these plants cause the disease known as trembles or alkali disease, and known especially to the medical profession as milksickness. Milksickness caused much trouble in pioneer days when the cows were allowed to graze in the open woods.

Rayless goldenrod has been found to be a cumulative poison. Animals affected by the poison become exceedingly weak, develop a ketosis, excrete acetone, become hyperglucemic, show a marked loss of weight and upon exercising show severe trembling. The latter symptom may be used for almost certain diagnosis.

Couch (2,3), by fractional extraction with solvents and animal testing, separated substances from rayless goldenrod and richweed which he thought to be identical and which were capable of reproducing trembles in sheep. He named this fraction tremetol, and made a chemical study of its properties.

Couch obtained tremetol from dry ground rayless goldenrod by extracting with alcohol (3). The solvent was distilled from the extract and the greenish fatty residue washed with

boiling water as long as anything dissolved. The insoluble material was thoroughly extracted with boiling 50% alcohol. the solvent was removed and the thick resinous mass was allowed to cool and harden, when the watery portion of the residue was poured off. The resinous mass was then thoroughly extracted with boiling 30% alcohol and the solution filtered hot from the insoluble matter. Upon evaporating the alcohol from the filtrate, an impure ester of tremetol and resin acids separated. The crude ester was hydrolyzed by boiling with 5% alcoholic potash for four hours, the alcohol was distilled off and the residue was dissolved in water. The free tremetol was extracted from this solution with successive portions of ether. The combined ether solutions were concentrated to convenient volume and washed first with dilute sodium hydroxide solution and then with water to remove possible phenols and resin acids. The purified ether solution was then mixed with four volumes of petroleum ether, filtered from any precipitate and the ether allowed to evaporate. The solution in ether and dilution with petroleum ether was always repeated at least twice to insure purity. On removal of the solvent, tremetol was obtained.

Tremetol was found to be a viscous oily liquid of pleasant aromatic odor. It did not solidify even at very low temperatures, and could not be made to crystallize. It appeared to have the characteristics of an alcohol; phenolic and alkoxy groups seemed to be absent. It did not react with phenylhydrazine nor with hydroxylamine, nor did it give color reactions with Schiff's reagent or ferric chloride. At the

temperature of boiling water tremetol was not decomposed, but it did decompose rapidly when attempts to distill it were made, even at pressures below 1 mm. It was found to be slowly volatile with steam although some alteration was thought to occur in the process. Although insoluble in water, acids and alkalies, it was found to be readily soluble in alcohol and the common organic solvents, less soluble in petroleum ether, but readily soluble in mixtures of that solvent with ether. Tremetol was found to oxidize slowly in air over a period of months, losing its characteristic odor and developing a rancid acetous odor.

Analytical figures and molecular weight determinations for tremetol indicated $C_{18}H_{32}O_8$ as the formula. Four atoms of bromine were absorbed per molecule at room temperature. Oxidation with potassium permanganate gave a white crystalline material, m.p. $181^{\circ}C$, not further investigated. Optical activity was found to be $\alpha_D^{30} -33.82^{\circ}$; density, $d_4^{25} 1.0787$; refractive index, $n_D^{30} 1.5345$.

EXPERIMENTAL

Rayless goldenrod (Aplopappus heterophyllus, Blake)^{1*} used in this investigation was gathered while the plant was in bloom, August 20, 1938, two miles east of Hagerman, New Mexico. The air-dried plant was baled and sent to Oklahoma A. & M. College, where it was ground in a small hammer mill. The total weight of air-dried meal after grinding was 225 pounds.

The general method of separation used was essentially that of Couch with some modifications. Hot extraction was employed instead of cold percolation of the meal.

The extraction equipment used consisted of a round copper can, 36 inches in height and 12 inches in diameter, fitted with a water condenser lid and carrying a screen bottomed copper basket to hold the meal. The lower two-thirds of the can was set into a covered 100-lb. lard can which was used as a steam jacket.

Ethyl alcohol was used as the extracting fluid. Five pounds of dry meal was soaked over night in a tin-lined ice cream can, with 95% alcohol. This amount was enough to fill the meal basket of the extractor.

The alcohol soaked meal was extracted for approximately six hours with 95% alcohol. The extracted meal, which had lost about 10% of its weight on the dry basis, was then

^{1*} The species was not identified by a botanical authority, but is the only toxic one reported to occur in the locality.

washed with water to recover the alcohol (which was in turn recovered, when sufficient had accumulated, by distillation in a ten gallon pot still with a punched screen column, to yield 85% alcohol). The recovered alcohol was used to supplement alcohol for soaking the meal.

After twenty pounds of dry meal had been extracted without removing the concentrate from the can, the cooled extract was filtered through glass wool^{2*} to remove the precipitated plant waxes.

As much alcohol as possible was distilled from the filtrate on a steam bath. Steam was then passed through the black gummy residue to remove all traces of alcohol and as much as possible of an essential oil.

This essential oil was extracted from the steam condensate with benzene. The benzene was then evaporated and the impure essential oil distilled in a Hickman Vacuum still at 100°C at approximately one millimeter pressure, to yield a light yellow oil of aromatic odor. The chemical properties of this oil are being studied. (1)

The gummy residue left after steam distillation was cooled and the water poured from it. The residue was then thoroughly extracted with 50% alcohol and the solution filtered hot through cotton^{3*} to remove any precipitate.

^{2*} Glass wool was used for this filter because it permitted both ease of filtering and yet adequate removal of the plant wax.

^{3*} Cotton was found to be an efficient filter for removing the sticky particles of precipitate without clogging too badly.

The alcohol was removed from the filtrate from the 50% extraction by distillation on a steam bath and finally by passing steam through the mixture as before. The residue was dissolved in 95% alcohol and then diluted with water to a 30% alcohol solution and was removed by hot filtration through cotton. This insoluble material was kept for future study.

Upon concentrating the filtrate from the 30% extract on a steam bath and removing all traces of alcohol with live steam as usual, a black residue again separated. After cooling, the water was poured off and the residue was hydrolyzed by boiling for four hours with 5% alcoholic potassium hydroxide, after which the alcohol was distilled off, the residue dissolved in water and extracted with successive portions of ethyl ether. The concentrated ether extract was washed with 5% sodium hydroxide to remove phenols and resin acids and then with water. The ether extract was then diluted with four volumes of petroleum ether, and the solution filtered to remove precipitated impurities. After evaporating the ether about 40 ml. of a viscous orange liquid remained. This liquid was divided into two parts so that distillation in the Hickman Vacuum still might be carried out in different ways for experimental purposes.

The first part of the orange liquid thus purified by Couch's method was placed in the Hickman still and the temperature raised to 100°C by means of a steam bath. When a pressure of one millimeter of mercury was maintained by the use of a Hyvac pump, a yellow oil began to distill from the liquid. The distillate was divided into four unequal portions

of 4, 3, 2.5, and 2 ml., collected over equal time periods of distillation.

The refractive indices of the four fractions at about 25° were as follows: (1) 1.5100; (2) 1.5132; (3) 1.5261; (4) 1.5025.

These fractions remained liquid for over a week; at that time fraction (2), after being accidentally shaken, deposited some white crystals. The other fractions were seeded with these crystals, fractions (1) and (3) producing similar crystals. Fraction (4) did not yield any crystals.

After the crystals in the first three fractions stopped growing in amount, the refractive indices of the mother liquids were again taken. They were as follows: (1) 1.5110; (2) 1.5156; (3) 1.5075; (4) 1.5025. These values, compared with the refractive indices before crystallization, might indicate that the remaining liquid in the four fractions was approaching a constant composition.

The crystals were collected, purified by recrystallization from petroleum ether, and kept for future study.

When the dark brown residue left on distillation was removed from the still with ether a chalk white precipitate formed. This was filtered from the ether soluble material and both of these materials were kept for future study.

The second portion of the crude 'tremetol' purified by Couch's method was placed in the Hickman still and the temperature maintained at 80° by means of a water bath. A one pound coffee can adapted with a hole in the side to act as a bath served the purpose very well. Approximately 6 ml. of a

light yellow oil distilled from the liquid under a pressure of one millimeter. The refractive index of this fraction was found to be 1.5222.

The temperature of the bath was raised to 100° and 5 ml. of a more viscous material was collected. The refractive index of this fraction was found to be 1.5238.

The temperature was then raised to 170° by means of a glycerine bath, and a waxy orange solid was distilled out. This fraction had lost the characteristic spicy aromatic odor of the other fractions. When the red glassy residue which remained in the Hickman still was removed with ether no white insoluble material separated, indicating that this substance might have distilled or decomposed at this temperature.

The fractions collected at 80° and 100° were seeded with crystals from those deposited by first distillates and both produced crystals, the 80° fraction in greater amount.

Since there was a large amount of precipitate from the 30% alcohol extraction of the 'tremetol', it was thought that it might still contain considerable quantities of the material, or materials, sought. This residue was, therefore, saponified in the same manner mentioned before for the 'tremetol' ester. A dark red jell was obtained from the hydrolyzed product after it was purified by the method used previously. However, this jell had the characteristic aromatic odor of 'tremetol', and on being distilled in the Hickman still gave a yellow oil similar in amount and properties to that obtained from the 'tremetol' ester. This oil was later combined with the previously distilled fractions. The entire amount of distilled

'tremetol' obtained from the 225 pounds of ground goldenrod did not exceed 40 ml. A determination of specific gravity of the oil gave a value of 0.9993 at 25°C.

Chemical tests and the preparation of derivatives were tried on both the undistilled and distilled parts of the jell, with the following results:

Urethan. α -naphthyl isocyanate was warmed with a small portion of the material. The small amount of precipitate formed was very high melting and was probably dinaphthyl urea formed by the reaction of α -naphthyl isocyanate with very small quantities of water that were present.

Picrate. An alcoholic saturated solution of picric acid was added to an alcoholic solution of the substance and brought to boiling. No precipitate formed when the solution was cooled.

Benzoate. A small portion of the substance was dissolved in pyridine and benzoyl chloride added. The solution was warmed and poured with stirring into water. The precipitate that formed was purified by reprecipitating from benzene and alcohol. The dried precipitate was partially crystalline, but melted at a low temperature to give a material which closely resembled the original substance.

Pyridine salt. No distinguishable salt was formed with pyridine.

Acetate. Acetylation with acetic anhydride and fused sodium acetate gave an orange precipitate which melted at a low temperature to give a material closely resembling the original substance.

Liebermann-Burchard reaction for sterols.^{4*} A chloroform solution of the substance was treated with a few drops of acetic anhydride and concentrated sulfuric acid added. The temperature was held at 15°-20°C. A rose-red color developed but upon standing for days it only became darker red, and did not exhibit the characteristic (rose-red through blue to green) series of colors found for typical sterols.

Carr-Price color reaction for carotinoids.^{5*} A small portion of the substance was added to an anhydrous chloroform solution of antimony trichloride. A green color developed. Carotinoids give a deep blue color when treated in this manner.

Phenylhydrazone of liquid fraction. Only a small part of the liquid reacted and gave a precipitate having an indefinite melting point at about 135°C.

Oxime. No precipitate formed.

The color reaction given by Couch as a means of testing for the presence of 'tremetol', using petroleum ether and sulfuric acid, was tried. The oil, jell, crystals, and essential oil all gave the cherry red color which Couch described as being specific for 'tremetol'.

A qualitative analysis of the distilled 'tremetol' gave no evidence of elements other than C, H and O.

Purification of the jell was attempted by chromatographic adsorption using petroleum ether as a carrier and magnesium

^{4*} Gilman, Henry, 'A Treatise of Organic Chemistry', John Wiley & Sons, New York, 1938, Vol. 2, p. 1272.

^{5*} Ibid., p. 1171.

oxide as an adsorbent. No separation was apparent.

Crystallization was attempted by lowering the temperature with dry ice and acetone. At low temperatures a hard brittle glass was produced without the formation of crystals, regardless of the presence or absence of a solvent. This substance melted to give the original material.

Investigation of crystals. The white crystals which were obtained from the distilled 'tremetol' fractions were recrystallized from petroleum ether and then from purified n-hexane to insure purity. The second recrystallization produced slender, needle shaped crystals which under the microscope appeared to exhibit twinning, and which had a sharp melting point at 84° - 84.5° C. The total amount secured did not exceed 100 mg.

Chemical tests with the crystals gave the following information as to their properties.

A small quantity of crystals dissolved in carbon tetrachloride absorbed several drops of bromine in the same solvent, thus indicating unsaturation.

A phenylhydrazone was made by the usual method and was found to have a melting point of 164° C after repeated recrystallization. No doubt the derivative isolated from the oil was an impure form of the same material.

The semicarbazone, similarly made and purified, melted at 203° - 204° C.

No solid oxime was obtained, although its preparation was attempted several times.

No reduction was noted when the crystals were treated with

ammoniacal silver nitrate, indicating that the compound is probably a ketone.

A sample of 'tremetol' separated by Couch in 1930, also from *Aplopappus heterophyllus*, was furnished through the courtesy of Dr. Couch. The undistilled sample had very much the same appearance as the material obtained in this investigation had before distillation. The odor was somewhat different and suggestive of a mixture of the essential oil and the lower fraction resulting from distillation of 'tremetol' as obtained in this work. After washing with 5% sodium hydroxide to remove possible acid decomposition products, the 'tremetol' sample was distilled in the Hickman still. A light yellow oil closely resembling the distilled oil of this investigation was obtained at 100°C. The residue in the still gave the same white, ether insoluble material as noted previously. The refractive index before distillation was 1.5390, and after distillation 1.5478. Although this distilled 'tremetol' was seeded with crystals obtained from a correspondingly distilled fraction of the material of this investigation, no crystal growth was noted. Instead, the crystals were dissolved upon standing.

DISCUSSION OF RESULTS

The hot extraction used in this investigation is considered to carry out the process more rapidly and with less mechanical work than cold percolation. Since in previous work the poison was not destroyed at temperatures of 100°C, this hot extraction seemed safe. It was further believed that the hot extraction would remove nothing which cold alcohol would not remove in time.

Since saponification of the residues from the 30% alcohol extraction in the separation of 'tremetol' yielded a quantity of 'tremetol' equal to that obtained in the usual way, it would appear that the 30% extraction might well be eliminated. Couch has suggested that the 50% alcohol extraction might be omitted. (4)

It is believed that the use of the Hickman Vacuum still allowed the 'tremetol', as obtained and purified by previous methods, to be distilled without the decomposition caused by the use of ordinary distillation equipment. No cracking should have occurred in the distillation at 100°C and one millimeter pressure, and certainly none at 80°C, since the material had been subjected repeatedly to these temperatures during extraction and purification. The white crystals are thus not considered to be a decomposition product. Another indication that cracking or decomposition had not occurred lies in the fact that one portion of the distillate at 100° was redistilled twice without leaving a residue.

It is suggested that, since the purification of 'tremetol' by diluting an ether solution with four volumes of petroleum

ether removed only a little material, this step might well be eliminated.

From the results of the distillation of 'tremetol' it appears that 'tremetol' is composed of several different parts, at least four of which have been separated. It is hoped that animal testing for the toxicity of the various parts, which is being carried out at this time (1), may define the toxic material still further.

No compound has been found in the literature which possesses the constants and derivatives found for the white crystals obtained from the distilled 'tremetol'. Hence it may be that a new compound has been isolated, probably an unsaturated ketone. A further investigation of these crystals to determine the structure of the compound would be of interest. A quantitative estimation of the number of ketone groups, quantitative analysis of some of the derivatives, crystallographic examination and absorption spectrum analysis would help considerably in the determination of the constitution of the compound.

It will be noted that the essential oil, the distilled 'tremetol' and the crystals all gave the red color suggested in a previous investigation as a specific qualitative test for 'tremetol'. Thus it is probable that this test is unreliable.

The refractive index of the 'tremetol' has been cited in previous work as a criterion of purity, but it will be noted from the results of this investigation that the index of refraction may vary over a wide range. Indeed, since it is

known that 'tremetol' is not one substance and since the parts may be separated in varying degrees of completeness, the index of refraction could not be expected to remain constant.

Treatment of the sample of 'tremetol' received from Dr. Couch according to the methods used in this investigation gave comparable results. The lack of crystal formation and the dissolving of the seed crystals may have been due to a small amount of essential oil in the sample which had not been removed. It is probable then that the material here isolated is approximately the same as the material obtained in the earlier investigation.

It has been stated by Couch that 'tremetol' is slowly volatile with steam, although some alteration was thought to occur in the process since there was a total loss of toxicity. It is suggested that if the essential oil had not all been removed from the 'tremetol', the volatile portion may have been essential oil. The essential oil has been found to give the 'tremetol' color test and hence might be mistaken for 'tremetol'. Since the essential oil is not toxic this might explain the 'loss of toxicity'.

SUMMARY

1. 'Tremetol' has been extracted from rayless goldenrod by continuous extraction with boiling ethanol. This method is believed to be simpler and more rapid than cold percolation.

2. Since a quantity of 'tremetol' was recovered from the residues of the 30% alcoholic extraction, this step in the purification might well be eliminated.

3. Contrary to the statements of Couch, 'tremetol' can be distilled without decomposition, at least in part. In a Hickman Vacuum still at about 1 mm. pressure, it was separated into the following fractions:

a. A yellow viscous distillate appearing at 80°-100°, having an aromatic odor, a density of 0.999 at 25°, and a refractive index of about 1.510 at 25°. No derivatives could be isolated.

b. A white crystalline unsaturated ketone which separated from fraction a, m.p. 84°C; phenylhydrazone, m.p. 164°C; semicarbazone, m.p. 203°-204°C.

c. A white ether-insoluble amorphous powder which does not distill at 100° but may do so at higher temperatures.

d. A semi-solid orange material distilling at 100°-170°, not having the spicy odor of the lower boiling fraction. No derivatives were obtained.

e. A dark red glassy solid residue, soluble in ether.

4. 'Tremetol' is accordingly not a pure compound, but a complex mixture. This has been verified by comparison of the

'tremetol' prepared in this work with a specimen of that isolated by Couch.

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AUTOBIOGRAPHY

I, Clarence A. Lathrop, was born on a farm south of Dexter, New Mexico, September 11, 1913. I attended the public schools of Dexter, graduating from Dexter Highschool in May, 1930. I attended New Mexico State College, State College, New Mexico, from September, 1931 to January, 1932 and from January, 1934 to June, 1937 at which time I received the degree of Bachelor of Science in Chemical Engineering. During the last two years at New Mexico State College, I worked as student chemist in the State Feed and Fertilizer laboratory.

In September, 1937, I entered the Graduate School of the Oklahoma Agricultural and Mechanical College, where I have worked for the past two years as a graduate assistant in the Chemistry department. I expect to receive the degree of Master of Science in May, 1939.

Typed by:

Katherine Lathrop