[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE GLIDDEN COMPANY, SOYA PRODUCTS DIVISION]

The Constituents of *Ceanothus Americanus*. I. Ceanothic Acid[1]

BY PERCY L. JULIAN, JOSEF PIKL AND RAY DAWSON

The plant *Ceanothus Americanus*, commonly called Jersey Tea, has attracted widespread attention for quite some time,[2] particularly because of the reported hemostatic action of a substance or certain substances present in the bark of the root. It is generally believed that the "active principle" in question is an alkaloid, but no satisfactory proof of this has yet been adduced.

Our work on the plant was begun in 1931 and was planned to include both basic and non-basic constituents. The ground bark of the root which we employed for the first extraction was purchased from R. J. Hilliers and Son, N. Y. The alcohol extract from 10 kg. of this sample yielded 12 g. of an acid, melting when pure at 354°. This substance occurs, therefore, in larger quantity than any other which hitherto has been isolated pure from the plant, and solely for this reason we have chosen to call it ceanothic acid.

We were surprised at the relative ease with which this acid was isolated in our first experiments, inasmuch as it had escaped the attention of all investigators. It was soon found, however, that our first specimen of ground bark had presented a fortunate sample, both from standpoint of yield and ease of isolation of the acid concerned.

In the summer of 1934, Mr. Dawson, who is a well-trained botanist, gathered rather large quantities of the root in the vicinity of Detroit, Michigan, and resumed the work which had been interrupted shortly after the first extractions referred to above. In addition to the material which he gathered, we have also employed ground bark of root generously donated by Flint, Eaton and Company of Decatur, Illinois. Material secured from each of the above-named sources yielded the acid in question. Its isolation in good yield, however, is not so simple a matter as we were first led to believe. Foremost among the difficulties involved are the sluggish crystallization of the acid when impure, and the presence in large quantity of an oil—apparently a fat—which hinders the crystallization of the acid under the conditions employed in our experiments.

Ceanothic acid may be classed among the so-called "bitter principles," its solutions in alkali having an extremely bitter taste. It crystallizes in beautiful, colorless lustrous needles from ether–petroleum ether or from methyl alcohol and may be purified by way of its crystalline barium salt. The acid seems to exist in two modifications with different melting points. All of our samples when first isolated melted at 354°. On long standing, the melting point of each of these samples was 339°, and was not altered by reconversion into the barium salt, recovery, and recrystallization.

Both high- and low-melting modifications yield the same results on analysis, and a series of such analyses, together with other data, points to the formula $C_{29}H_{44}O_5$ for the acid.

In aqueous-alcoholic solution ceanothic acid titrates with barium hydroxide as a dibasic acid, and this affords a convenient method for determining its molecular weight, the values found being in good agreement with the formula proposed.

The acid contains no phenolic group, but does contain one hydroxyl group in addition to the two carboxyl groups. It yields a lactone on heating just above its melting point, losing exactly one mole each of water and carbon dioxide. One of the carboxyl groups must therefore be in the gamma position with reference to the hydroxyl group. The lactone melts at 234° and sublimes, without decomposition, in high vacuum. Analyses of it indicate the formula $C_{28}H_{42}O_2$, thus further supporting our proposed formula for the acid.

Ceanothic acid cannot be esterified with alcoholic hydrochloric acid in the usual manner but is readily converted into a crystalline ester, m. p. 222°, with diazomethane. The carbon-hydrogen analyses agree with the expected formula, $C_{31}H_{48}O_5$, and methoxyl determinations show the presence of two methoxyl groups. This

---

(1) This work, which was completed for the most part while the authors were associated with De Pauw University, is abstracted in large part from the Senior Researches of Ray Dawson at the Institution. The several papers published from this Institution by the senior authors of this paper and their undergraduate students represented a part of a program initiated and courageously supported by Dean W. M. Blanchard in his attempt to "bridge the gap between college and university." Mr. Dawson is now Fellow in Botany at Yale University.

(2) For summary of literature, see Bertho and Liang, *Arch. der Pharm.*, **271**, 273–276 (1933).

ester stubbornly resists saponification, long boiling with acids and alkali having apparently no effect.

That the hydroxyl group is still present in the ester is proved by acetylation of the latter with acetic anhydride. This acetyl compound, m. p. 157°, analyzes, as would be expected, for a substance of the composition $C_{33}H_{50}O_6$. The ester was recovered from it by saponification with alcoholic sulfuric acid. Attempts to acetylate or benzoylate the acid were unsuccessful, no crystalline product being isolated.

Oxidation of ceanothic acid either with chromic acid or with potassium permanganate yielded only intractable gums which could not be induced to crystallize.

Ceanothic acid is not the active hemostat of the alcohol extract of *Ceanothus Americanus*. Solutions of the acid were prepared by dissolving it in 0.1 *N* sodium hydroxide and adding 0.05 *N* hydrochloric acid almost to precipitation. The *p*H of such solutions was approximately 8.0. They were diluted until every cc. represented 50 mg. of acid and injected intravenously in doses of from 100 to 300 mg. On several animals employed (dogs) the normal coagulation time was not decreased.

The authors are indebted to Professor Julian H. Lewis of the Department of Pathology of the University of Chicago for the tests on the hemostatic action of the acid. They are also indebted to Flint, Eaton and Company of Decatur, Illinois, for large supplies of material, and to the Rosenwald Fund and the Omega Psi Phi Fraternity for generous grants which made possible the completion of this first work on this very interesting plant.

Experimental Part

Isolation of Ceanothic Acid.—Best results were obtained when the ground bark of the root was treated with alcohol in an ordinary pharmaceutical percolator. Extraction in a continuous extractor with alcohol at elevated temperatures seems to give rise to contamination of the acid with impurities which prevent ready crystallization. The alcohol was removed from the extract by distillation *in vacuo*, the residue dried, powdered, mixed with sand, and stirred with ether until test showed that ether removed no further material. To the concentrated ether extract, petroleum ether was added just to turbidity, and the material placed in the ice chest. Sufficient additional petroleum ether to produce turbidity was added every few days, and after about three weeks the acid had crystallized, yield from 10 kg., 12 g.; recrystallized from ether–petroleum ether, or when fairly pure from methyl alcohol, m. p. 354°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.68; H, 9.38. Found: C, 73.95, 73.40; H, 9.63, 9.46.

A sample of the acid thus obtained was allowed to stand in a closed container for a year. It then melted at 336°. Converted into the crystalline barium salt, recovered, and recrystallized from ether–petroleum ether, the m. p. was 339°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.68; H, 9.38. Found: C, 73.68, 73.60; H, 9.56, 9.69.

Several samples of highly purified acid, on first isolation, melted at 354°. Invariably on standing for some time they gave the lower melting point of 339°.

A weighed quantity of the acid, dissolved in ethyl alcohol, was titrated with 0.4 *N* barium hydroxide. The equivalent weights found were 251.1, 248.5 and 247, giving an average molecular weight by this method of 498 against a calculated value of 472. The barium salt separated from the alcohol–water mixture.

Ceanothic acid contains no nitrogen.

Lactone of Ceanothic Acid.—2.0466 grams of ceanothic acid was heated to 340–350° in a gentle current of pure nitrogen, and the carbon dioxide and water absorbed in the apparatus ordinarily employed in organic macro combustions for carbon and hydrogen. The water weighed 0.0865 g. and the carbon dioxide 0.1735, the total loss in weight being 12.70%; calculated, 13.14%.

The lactone was purified by recrystallization from low boiling petroleum ether, followed by sublimation in high vacuum, m. p. 234°.

Anal. Calcd. for $C_{28}H_{42}O_2$: C, 81.89; H, 10.31. Found: C, 81.50, 81.70; H, 10.80, 10.80.

Dimethyl Ester of Ceanothic Acid.—To a solution of 1 g. of acid in 50 cc. of absolute ether, an ethereal solution of diazomethane from 5 cc. of nitrosomethylurethan was added. The solution which immediately evolved gas was allowed to stand in a dark place for several hours. On evaporation of the ether, a crystalline residue remained. This was taken up in ether, petroleum ether added to precipitate a little flocculent material, the solution filtered and concentrated. After another recrystallization from ether–petroleum ether, the m. p. was 223°. Analyses were carried out on samples purified in this manner and also on samples distilled in high vacuum; both gave essentially the same results.

Anal. Calcd. for $C_{31}H_{48}O_5$: C, 74.40; H, 9.60; OCH_3, 12.39; mol. wt., 500. Found: C, 74.32, 74.30; H, 9.74, 9.77; OCH_3, 12.35;[3] mol. wt. (Rast), 525.

Acetyl Derivative of the Ester.—A solution of 0.8 g. of the dimethyl ester of ceanothic acid in 10 cc. of acetic anhydride was heated on the water-bath for three hours. After removal of the anhydride in vacuum, a little ether was added and last traces of anhydride removed again under vacuum. The residue was dissolved in low boiling petroleum ether (38°) and the solution concentrated; recrystallized from low-boiling petroleum ether, m. p. 157°; yield 0.62 g.

Anal. Calcd. for $C_{33}H_{50}O_6$: C, 73.01; H, 9.29. Found: C, 73.11, 73.08; H, 9.37, 9.41.

A solution of 0.1 g. of the acetyl compound in 20 cc. of 20% sulfuric acid and 5 cc. of ethyl alcohol was boiled

---

(3) Method of Viebock and Schwappach, *Ber.*, **63**, 2818 (1930).

for six hours, allowed to cool down, the crystals filtered and recrystallized from ether–petroleum ether, m. p. 219°. A mixed melting point with the ester showed no depression. Attempted saponification of the acetyl compound with alcoholic potassium hydroxide gave no crystalline product.

Summary

A dicarboxylic acid, which yields a lactone on heating above its melting point, has been isolated from the bark of the root of *Ceanothus Americanus*. From it a crystalline lactone and dimethyl ester, and from the latter a crystalline acetyl derivative, have been prepared. Examination of the acid and these derivatives points to the formula, $C_{29}H_{44}O_5$ for the former, which it is proposed to call ceanothic acid.

CHICAGO, ILLINOIS RECEIVED NOVEMBER 1, 1937

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF SCHERING CORPORATION]

Preparation of *epi-allo*-Pregnanol-3-one-20

BY G. FLEISCHER, B. WHITMAN AND E. SCHWENK

Marker, *et al.*[1] isolated *epi-allo*-pregnanol-3-one-20 from the urine of pregnant women and have prepared this substance both from 3-chloro-*allo*-cholanic acid[2] and from *allo*-pregnanediol.[3] We have prepared this substance from pregnenolone, which is now made on a technical scale. Thus larger amounts of *epi-allo*-pregnanolone may be secured easily. Our substance had a higher melting point and lower rotation than that originally reported by Marker and co-workers.[1]

Experimental

Preparation of *allo*-Pregnanedione.—Pregnenolone was reduced with platinum oxide and hydrogen in alcoholic solution to *allo*-pregnanolone, and this oxidized with chromic acid in acetic acid solution to *allo*-pregnanedione in the known manner.[4]

Preparation of *epi-allo*-Pregnanolone.—Platinum oxide (0.5 g.) in 15 cc. of acetic acid was reduced to platinum black, a hot solution of 6.75 g. of *allo*-pregnanedione in 40 cc. of acetic acid and 0.55 cc. of 48% hydrobromic acid were added, and the whole was shaken with hydrogen, while being heated. During one and one-half hours one mol of hydrogen had been taken up. After filtering from the catalyst, the solution was poured into water and taken up in ether, and the extract was washed with sodium hydroxide and water. After the ether had been distilled, the residue was boiled with 25 cc. of 2% potassium hydroxide in methanol for two hours to hydrolyze any acetate which was formed during reduction, and then poured into water. The white precipitate was filtered off, washed with water, dried, and dissolved in 300 cc. of 90% alcohol, and a solution of 10 g. of digitonin in 1 l. of 90% alcohol was added. After standing for one-half hour a precipitate settled, and this was collected, washed thoroughly with alcohol, and dried; yield, 7.8 g. (corresponding to 1.6 g. of *allo*-pregnanolone). The alcoholic solution was evaporated to a very small volume, mixed with water, and the *epi-allo*-pregnanolone extracted with ether. The undissolved digitonin was filtered off and washed thoroughly with ether; dry weight, 3.8 g. The ether was washed twice with water, then evaporated. On crystallizing the crude material from alcohol, 3 g. of crude *epi-allo*-pregnanolone was obtained, having a melting point of 147–152°, $[\alpha]_D$ +58° in absolute ethanol. After several recrystallizations, the melting point was constant at 176–178°;[5] $[\alpha]_D$ +87.7° in absolute ethanol.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.18; H, 10.77. Found: C, 78.92; H, 10.77.

Acetate of *epi-allo*-Pregnanolone.—*epi-allo*-Pregnanolone (100 mg.) was boiled with acetic anhydride (2 cc.) for thirty minutes, anhydride was then evaporated *in vacuo*, and the product recrystallized several times from petroleum ether and aqueous alcohol. The melting point was 141–142°, its rotation $[\alpha]^{22}_D$ + 94.5° in absolute ethanol.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.61; H, 10.07. Found: C, 76.72; H, 10.01.

Splitting of the Digitonide.—The 7.8 g. of digitonide was dissolved in 125 cc. of dried pyridine and, while stirring, 1.3 l. of dry ether was dropped in during one hour. The digitonin was filtered off (dry weight 6 g.), and the filtrate was slightly acidified, washed, dried and the ether evaporated. The residue was *allo*-pregnanolone[6] (1.5 g.), m. p. 178–185°. After several recrystallizations the substance melted at 194°; $[\alpha]^{22}_D$ +90.5° in absolute ethanol.

Acetate of *allo*-Pregnanolone.—*allo*-Pregnanolone, acetylated in the usual manner, gave material melting at 144° (uncorr.); $[\alpha]^{22}_D$ +79.8° in absolute ethanol.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.61; H, 10.07. Found: C, 76.71; H, 10.11.

Summary

The preparation of *epi-allo*-pregnanol-3-one-20 from pregnenolone is described. *allo*-Pregnanolone was obtained as a by-product.

BLOOMFIELD, N. J. RECEIVED NOVEMBER 16, 1937

---

[1] Marker, Kamm and McGrew, THIS JOURNAL, **59**, 616 (1937).

[2] Marker, *et al.*, *ibid.*, **59**, 1367 (1937).

[3] Marker, *etal.*, *ibid.*, **59**, 1595 (1937).

[4] A. Butenandt and G. Fleischer, *Ber.*, **68**, 2094 (1935).

[5] We wish to thank Dr. Marker for the kindness of having made a mixed melting point determination of our substance and his own *epi-allo*-pregnanolone. There was no depression. Dr. Marker now finds also the higher melting point for his own preparation.

[6] A. Butenandt and L. Mamoli, *Ber.*, **67**, 1897 (1934).