[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY]

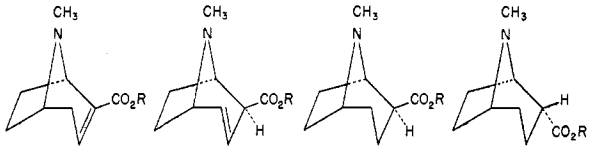
The Structure of Anhydroecgonine Ethyl Ester

BY STEPHEN P. FINDLAY

RECEIVED AUGUST 18, 1952

Anhydroecgonine ethyl ester contains an ethylenic linkage in conjugation with its carbethoxy group as indicated by its catalytic reduction to two isomeric derivatives and by its infrared absorption spectrum.

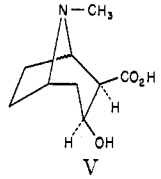
When anhydroecgonine ethyl ester (I, R = C_2H_5) in alcoholic solution is hydrogenated in the presence of palladized charcoal, one molecule of hydrogen is absorbed per molecule of amino ester, and a product is obtained consisting of the ethyl esters of the two diastereoisomeric hydroecgonidines (dihydroanhydroecgonines) (III and IV, R = C_2H_5). These were isolated by treating the prod-



I II III IV

uct with the stoichiometric quantity of picric acid and separating the isomeric picrates by fractional crystallization. One picrate, obtained in relatively very small yield, is liquid; the other is crystalline and melts at 113–114°. The crystalline picrate has the composition of hydroecgonidine ethyl ester picrate and generates this ester on appropriate treatment. The ester furnishes a crystalline oxalate and a chloroaurate melting at 177.5–181°. The latter derivative was obtained, though in apparently less pure condition, as hereafter explained, by von Braun and Müller[1] and later by Gadamer and John.[2] Pseudohydroecgonidine ethyl ester, recovered from the liquid picrate gives no crystalline oxalate but does yield a chloroaurate melting at 122.5°. This ester is presumably identical with Willstätter's hydroecgonidine ethyl ester,[3] which was obtained by esterifying the product resulting from the reduction of anhydroecgonine with sodium and amyl alcohol and which gave a liquid picrate and a chloroaurate melting at 121–122°.

Anhydroecgonine (I or II, R = H) is prepared by dehydrating the β-hydroxy acid, ecgonine (V),[4] with boiling phosphorus oxychloride[5] or boiling strong hydrochloric acid.[4] Only one of several conceivable isomers is ever obtained from these re-



V

actions, and this is apparently not isomerized by heating at 100° with concentrated aqueous potassium hydroxide[6] although similar treatment of analogous aliphatic and alicyclic compounds affords mixtures of α,β- and β,γ-unsaturated acids.[7] The absence of an isomer of anhydroecgonine is undoubtedly owing to its bridged ring system. While there is no way of knowing *a priori* whether anhydroecgonine ethyl ester has structure I or II (R = C_2H_5), the latter appears improbable and, hydrogenated under the conditions employed in this investigation, could hardly have resulted in more than one product. It is therefore concluded that the starting material has the structure assigned (R = C_2H_5), and it seems reasonable to infer also that anhydroecgonine itself and eccaine[1,8] contain the α,β-location of the double bond.

A comparison of the infrared absorption spectra of the ethyl esters of anhydroecgonine and hydroecgonidine leads to the same conclusion (Fig. 1). The α,β-position of the double bond in I is strongly indicated because (1) the intense carbonyl absorption band, which is located in the normal range for the carboethoxy group, is shifted 0.07 μ in the direction of longer wave lengths as would be expected for a conjugated ester[9] and (2) the absorption of the ethylenic linkage is much stronger than would be expected for an unconjugated endocyclic ethylenic linkage.[10] The β,γ-position of the double bond is unlikely because (1) any shift in the carbonyl absorption band would be opposite to that observed and (2) the band at 6.10 μ is too strong for an isolated endocyclic ethylene linkage.[10] The presence of a cyclopropane ring structure is excluded because it cannot account for the absorption at 6.10 μ.[11,12]

(1) J. v. Braun and E. Müller, *Ber.*, **51**, 235 (1918).

(2) J. Gadamer and C. John, *Arch. Pharm.*, **259**, 227 (1921).

(3) R. Willstätter, *Ber.*, **30**, 702 (1897).

(4) This structure is speculative. However, in view of the isomerization of ecgonine to pseudoecgonine in strong alkali (A. Einhorn and A. Marquardt, *ibid.*, **23**, 468 (1890)), it appears that the two compounds are epimeric at the α-carbon rather than at the hydroxyl-bearing carbon as supposed by Willstätter and Bommer. Ecgonine undergoes dehydration with hot hydrochloric acid (A. W. K. de Jong, *Rec. trav. chim.*, **56**, 186 (1937)) much more readily than pseudoecgonine and ecgonine methyl ester methiodide Hofmann elimination (R. Willstätter and M. Bommer, *Ann.*, **422**, 15 (1921)) much more readily than the pseudo isomer. Recent views on the mechanism of elimination reactions (M. L. Dhar, E. D. Hughes, C. K. Ingold, A. Mandour, G. A. Maw and L. I. Woolf, *J. Chem. Soc.*, 2093 (1948)) suggest, but do not prove, that the latter, and possibly the former, decomposition proceeds in a *trans* manner.

(5) A. Einhorn, *Ber.*, **20**, 1221 (1887).

(6) J. Gadamer and T. Amenomiya, *Chem. Zentr.*, I, **75**, 721 (1904).

(7) (a) G. A. R. Kon, E. Leton, R. P. Linstead and L. G. B. Parsons, *J. Chem. Soc.*, 1411 (1931); (b) E. J. Boorman and R. P. Linstead, *ibid.*, 258 (1935).

(8) (a) P. Karrer, "Lehrbuch der organischen Chemie," Rascher Verlag, Zürich, 1948, p. 905; (b) I. Heilbron, "Dictionary of Organic Compounds," Vol. II, Oxford University Press, New York, N. Y., 1946, p. 1.

(9) H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949, chart facing p. 20.

(10) R. N. Jones, P. Humphries, E. Packard and K. Dobriner, THIS JOURNAL, **72**, 86 (1950).

(11) M. Josien, N. Fuson and A. S. Cary, *ibid.*, **73**, 4445 (1951).

(12) G. Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules," D. Van Nostrand Co., Inc., New York, N. Y., 1945, p. 352.

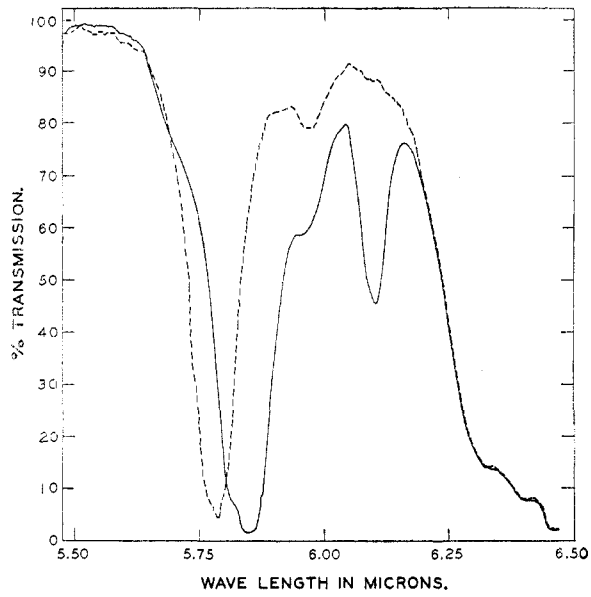


Fig. 1.——, Anhydroecgonine ethyl ester in carbon tetrachloride solution; -------, hydroecgonidine ethyl ester in carbon tetrachloride solution.

While the results of this investigation are in agreement with conclusions reached earlier, the latter have been based on experimental evidence which is ambiguous or untrustworthy. The objections to Willstätter's original proposal (II, R = H) for anhydroecgonine[13] have been discussed by de Jong.[14] The fallacy in the reasoning which induced Willstätter to abandon structure II for structure I[15] has been pointed out by Gadamer and John.[2]

von Braun and Müller[1] hydrogenated anhydroecgonine ethyl ester in a manner similar to that used in this undertaking. After distillation, their product, which furnished a chloroaurate melting at 167° rather than at 177.5–181°, was tacitly assumed to be pure hydroecgonidine ethyl ester. However, they found also that a mixture of hydroecgonidine ethyl ester and its pseudo isomer, prepared by Willstätter's method,[3] cannot be resolved by distillation. It is probable, therefore, that their hydroecgonidine ethyl ester likewise contained a small amount of the pseudo isomer.

As evidence for structure I Gadamer and John later demonstrated that anhydroecgonine (I, R = H) was catalytically reduced to two different hydroecgonidines (III and IV, R = H).[2] These they converted to the ethyl ester chloroaurates which melted at 122° and 173–174°. The higher-melting derivative should be identical with von Braun and Müller's chloroaurate, but they did not note this. To have done so should have entailed a remark on the rather large discrepancy in the melting points. Since there is a similar discrepancy in melting points between Gadamer and John's higher-melting chloroaurate and that reported here, it appears that their preparation, as well as von Braun and Müller's, was impure. The work of Gadamer and John contains other errors both of omission and commission, and their conclusions, confirmed here, have usually been ignored by reviewers.[16]

According to de Jong,[14] the much readier dehydration of ecgonine compared to pseudoecgonine in strong hydrochloric acid to give anhydroecgonine would be surprising if the ensuing double bond were created in the β,γ-position (structure II, R = H) but is understandable on the assumption that the β-hydroxyl group of ecgonine is *trans* to the carboxyl permitting *cis* elimination of water, which he conceives to be easier than *trans* elimination.

Acknowledgment.—The writer is indebted to Mr. Marvin H. Rowe of this Institute for the infrared measurements and their interpretation and also to the Institute's Microanalytical Laboratory, directed by Dr. William C. Alford, for the analytical data.

Experimental[17]

Infrared Absorption Measurements.—Spectra were taken on a Perkin-Elmer model 21 spectrometer with NaCl optics without compensation for solvent absorption. The solutions were prepared by diluting 100 mg. of substance to 10.0 ml. with carbon tetrachloride. A cell thickness of 0.58 mm. was used.

Anhydroecgonine Hydriodide.—A mixture of impure ecgonine (m.p. 199–204°) (20 g.) and phosphorus oxychloride (100 ml.) was boiled one hour and the mixture worked up according to the directions of Einhorn[5]: 21.3 g. (67%) of crude hydriodide. Purified from alcohol it consisted of yellowish crystals, m.p. 200–205°. Treated with silver oxide it furnished **anhydroecgonine** which was purified from methanol: rhombic crystals, m.p. 234° (dec.).

Anhydroecgonine Ethyl Ester.—A mixture of anhydroecgonine hydriodide (18.4 g.), a nearly 100% excess of freshly prepared silver chloride and water (100 ml.) was shaken overnight. Anhydroecgonine hydrochloride was recovered by filtering off the silver halides and evaporating the filtrate to dryness *in vacuo*. It was esterified by treating with about eight times its weight of 10% absolute alcoholic hydrochloric acid and refluxing gently one hour. Processed according to Willstätter's directions[3] for hydroecgonidine ethyl ester, anhydroecgonine ethyl ester (9.4 g., 77%) was obtained: b.p. 120° (10 mm.), d^{20}_4 1.061 (reported[18] d^{21}_4 1.064), n^{20} 1.4952 (reported[2] $n^{11.7}D$ 1.49930).

The picrate, prepared in and purified from alcohol, consisted of yellow needles, m.p. 169–170° (reported[18] 168°).

Catalytic Reduction of Anhydroecgonine Ethyl Ester.—A mixture of anhydroecgonine ethyl ester (11.6 g., 0.0595 mole), alcohol (600 ml.) and 5% palladized charcoal (6.00 g.) absorbed 1400 ml. (0.0625 mole) of hydrogen (S.T.P.), 105% of the theoretical quantity. The catalyst-free solution was fractionally distilled to about 100 ml. of residue which was treated with 90% picric acid (15.1 g.) dissolved in hot absolute alcohol (100 ml.). From a mixture of this composition the picrate separated first as a liquid which slowly crystallized. When more absolute alcohol (150 ml.) was added, no liquid picrate separated as the solution cooled. **Hydroecgonidine ethyl ester picrate** precipitated as hexagonal prisms, m.p. 112–114°, 18.1 g. (71%). Recrystallized from absolute alcohol the salt melted at 113–114.5°. No liquid picrate appeared to be formed during recrystallization.

Anal. Calcd. for $C_{17}H_{22}N_4O_7$: C, 47.9; H, 5.21; N, 13.1. Found: C, 47.9; H, 5.05; N, 12.9.

Fractional crystallization of the mother liquors furnished 1–2 g. of less pure picrate and about 3 g. of a liquid picrate.

Hydroecgonidine ethyl ester was liberated from the picrate (m.p. 113–114.5°) by treating with excess normal sodium hydroxide and extracting with ether. The ester was recovered and purified by two vacuum distillations to a water-white liquid having a faint odor reminiscent of pyridine: b.p. 122° (14 mm.), d^{20}_4 1.0388, $n^{20}D$ 1.4758 (reported[2] b.p. 132–134° (13 mm.), d^{20}_4 1.041, $n^{20.5}D$ 1.47805).

(13) R. Willstätter and W. Müller, *Ber.*, **31**, 2659 (1898).
(14) A. W. K. de Jong, *Rec. trav. chim.*, **56**, 198 (1937).
(15) R. Willstätter and M. Bommer, *Ann.*, **422**, 22 (1921).
(16) *Cf.* T. A. Henry, "The Plant Alkaloids," 4th Edition, The Blakiston Co., Philadelphia, Pa., 1949, p. 99; also ref. 8a, p. 902.
(17) All melting points are corrected.
(18) C. Liebermann, *Ber.*, **40**, 3602 (1907).

Mixed with an equimolar quantity of oxalic acid in absolute alcohol, it yielded **hydroecgonidine ethyl ester binoxalate** which was purified from the same solvent: irregular prisms, m.p. 143°.

Anal. Calcd. for $C_{13}H_{21}NO_6$: C, 54.4; H, 7.39. Found: C, 54.2; H, 7.25.

When the ester (165 mg.) was added to a solution of chloroauric acid (330 mg.) in alcohol (1.5 ml.), **hydroecgonidine ethyl ester chloroaurate** precipitated. It was purified from alcohol: platelets some of which appeared to be parallelopipeda. In a bath the temperature of which was rising at a rate of less than a degree per minute this derivative melted at 177.5–181° (reported[1,2] 167°, 173–174°).

The liquid picrate, treated in the same manner as the crystalline isomer, furnished impure **pseudohydroecgonidine ethyl ester** (1.1 g.). Combined with an equimolar quantity of oxalic acid in absolute alcohol, the foregoing oxalate (m.p. 143°) crystallized slowly. The non-crystalline portion was mixed with dilute alkali and extracted with ether. The recovered ester was treated in dry ether solution with dry hydrogen chloride, but the liquid hydrochloride which separated did not crystallize. Regenerated once more the ester (0.50 g.) was added to a mixture of chloroauric acid (0.50 g.) and alcohol (1.5 ml.). The ensuing precipitate (m.p. 119°) was crystallized to constant melting point (three times) from alcohol: short, yellow prisms, m.p. 122.5° (reported[1,3] 122°).

BETHESDA, MD.

[CONTRIBUTION NO. 1746 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

An Agent from *E. coli* Causing Hemorrhage and Regression of an Experimental Mouse Tumor. III. The Component Fatty Acids of the Phospholipide Moiety[1]

BY MIYOSHI IKAWA, J. B. KOEPFLI, S. G. MUDD AND CARL NIEMANN[2]

RECEIVED OCTOBER 22, 1952

Lauric acid, myristic acid, palmitic acid and an optically active β-hydroxymyristic acid have been isolated from hydrolysates of the phospholipide moiety of the hemorrhagic agent obtained from *E. coli*. The optically active β-hydroxymyristic acid appears to possess the D-configuration.

The agent which is synthesized by *E. coli* and which produces a hemorrhagic response in and causes the regression of the experimental mouse sarcoma 180 has been shown to be a complex polysaccharide which contains both a peptide and a phospholipide component.[3] It is the purpose of this communication to comment upon the nature of the phospholipide moiety particularly in respect to its component fatty acids.

When the hemorrhagic agent was subjected to partial hydrolysis, with aqueous sulfuric acid, a fraction was obtained that was insoluble in water but which was soluble in chloroform. This chloroform-soluble fraction was further fractionated into acetone-soluble and acetone-insoluble fractions. The latter fraction was found to contain phosphorus and, from its solubility behavior, may be classified as a phosphatide. Hydrolysis of the phosphatide, *i.e.*, the chloroform-soluble and acetone-insoluble fraction, with aqueous hydrochloric acid and subsequent extraction of the hydrolysate with ether gave an ether-soluble fatty acid fraction.[4]

When an ethereal solution of the fatty acid fraction was triturated with ligroin an optically active acid, m.p. 73–74°, was obtained. It was shown that this compound had the molecular formula $C_{14}H_{28}O_3$, that it contained, in addition to the carboxyl group, a hydroxyl group, and probably only one terminal C-methyl group. The failure of the hydroxy acid to react with lead tetraacetate indicated that the compound was not an α-hydroxy acid.[5] Reaction of the hydroxy acid with thionyl chloride followed by hydrogenolysis gave myristic acid, and when the hydroxy acid was oxidized with alkaline permanganate, lauric acid was obtained. These data suggested that the acid of m.p. 73–74° was one of the optical isomers of β-hydroxymyristic acid. The synthesis of β-hydroxymyristic acid has been described[6,7] but apparently no attempt has been made to resolve the synthetic acid. Therefore β-hydroxymyristic acid was prepared, from lauraldehyde and ethyl bromoacetate, and resolved into its optical isomers with the aid of *d*-α-methyl-β-phenylethylamine. The *l*-acid obtained from the less soluble salt proved to be identical with the naturally occurring acid.

There is no record of the previous isolation of β-hydroxymyristic acid from a natural source. However Bergström, Theorell and Davide[8] have reported that *l*-β-hydroxydecanoic acid may be obtained from a metabolic product of *Pseudomonas pyocyanes*, and Jarvis and Johnson[9] isolated the same compound from the hydrolysate of a glycolipide obtained from *Ps. aeruginosa*. Furthermore *d*-β-hydroxycaproic and *d*-β-hydroxycaprylic acid have been obtained as hydrolysis products of the glycolipids produced by the corn smut *Ustilago zeae*,[10] and the mycolic acids from the human tubercle and the diphtheria bacillus are believed to be β-hydroxy acids.[11,12] From these observations it

(1) Supported from 1938 to 1943 by grants from the Argonaut Foundation and from 1948 onwards by grants from the National Cancer Institute of the U. S. Public Health Service.

(2) To whom inquiries regarding this article should be sent.

(3) M. Ikawa, J. B. Koepfli, S. G. Mudd and C. Niemann, *J. Nat. Cancer Inst.*, **13**, 157 (1952).

(4) The other fractions obtained at this stage, *cf.* Experimental section, were reserved for an investigation of the nitrogenous components of the phosphatide. The results of this study will be described in a separate communication.

(5) R. Criegee, "Newer Methods of Preparative Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1948.

(6) H. Thaler and G. Geist, *Biochem. Z.*, **302**, 369 (1939).

(7) F. L. Brensch, *Fortschr. Chem. Forsch.*, **1**, 567 (1950).

(8) S. Bergström, H. Theorell and H. Davide, *Arch. Biochem.*, **10**, 165 (1946); *Arkiv. Kemi, Mineral., Geol.*, **23A**, No. 13 (1946).

(9) F. G. Jarvis and M. J. Johnson, THIS JOURNAL, **71**, 4124 (1949).

(10) R. U. Lemieux, *Can. J. Chem.*, **29**, 415 (1951).

(11) J. Asselineau and E. Lederer, *Biochem. Biophys. Acta*, **7**, 126 (1951).

(12) E. Lederer, V. Portelance and K. Serck-Hanssen, *Bull. soc. chim.*, [5] **19**, 413 (1952).