tanone, similarly prepared, had m. p. 155°; $[\alpha]_D$ +40.6°; mixed m. p. 155°.

5,6-Dihydrostigmastanone oxime, recrystallized from aqueous alcohol, melted at 210°.

Anal. Calcd. for $C_{29}H_{51}ON$: C, 81.0; H, 12.0. Found: C, 80.8; H, 12.1.

Stigmastanone oxime had m. p. 210°; mixed m. p. 210°.

Ozonization of 5,6-Dihydrostigmasteryl Acetate.—One gram of the sterol acetate was suspended in 10 ml. of glacial acetic acid which had been freed of aldehydes by distillation over chromic oxide. Ozone in excess was passed through the suspension for one hour. The clear solution was added to 100 ml. of water and the mixture distilled to one-quarter the volume into a suspension of 800 mg. of 2,4-dinitrophenylhydrazine in 100 ml. of 95% alcohol containing 1 ml. of concentrated hydrochloric acid. The mixture of the hydrazone and unchanged reagent was washed with water and dried. It was suspended in benzene and percolated through a column of activated alumina. Benzene was used to elute the yellow hydrazone, while the dinitrophenylhydrazine reagent remained strongly adsorbed at the top of the column. The benzene solution was evaporated to dryness; the residue, recrystallized several times from alcohol, yielded 100 mg. of a yellow crystalline compound; m. p. 109°; $[\alpha]_D$ 0°.

Anal. Calcd. for $C_{13}H_{18}N_4O_4$: C, 53.02; H, 6.19; N, 19.03. Found: C, 52.93; H, 6.13; N, 18.95.

Stigmasterol was treated in the same manner and the dinitrophenylhydrazone isolated; m. p. 121°; $[\alpha]_D$ −5.8° in benzene; mixed m. p. 119°.

In another ozonization of 250 mg. of the sterol acetate, some of the volatile aldehyde, collected as such, displayed a positive fuchsin test.

The author would like to express his appreciation for the help and guidance given by Dr. H. T. Clarke; and is indebted to Mr. W. Sascheck for the microanalyses reported in this paper.

Summary

The sterols of a fresh-water sponge, *Spongilla lacustris*, were separated by means of repeated chromatographic adsorption on activated alumina. One fraction, strongly adsorbed, yielded an impure sterol with conjugated double bonds, displaying an absorption spectrum similar to that obtained with ergosterol. The fraction less strongly adsorbed by the alumina yielded a pure monounsaturated sterol identified as 5,6-dihydrostigmasterol.

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[CONTRIBUTION FROM THE U. S. BUREAU OF NARCOTICS LABORATORY]

Isolation of Ecgonidine Methyl Ester from Coca Seeds[1]

BY JOHN R. MATCHETT AND JOSEPH LEVINE

Although a copious literature exists regarding the alkaloids of the coca leaf, nothing appears to have been reported regarding the chemistry of the seeds. In this Laboratory we have had occasion to examine the seeds of two varieties, *Erythroxylon coca*, Lam., obtained from Peru and *Erythroxylon novogranatense*, (Morris) Hieron, obtained from Java, and have isolated from each an alkaloid which proved to be the methyl ester of ecgonidine. Neither ecgonine nor its esters were present in amounts sufficient for identification.

Ecgonidine and certain of its derivatives are well known, though the methyl ester does not appear to have been described previously. The ethyl ester has been isolated in small yield from the by-alkaloids of the coca leaf by Liebermann,[2] who expressed the opinion that it did not occur there naturally, but was probably formed from ecgonine during the long processing required to separate the ecgonine alkaloids.

The conditions under which ecgonidine methyl ester was isolated from the seeds were sufficiently mild to ensure against decomposition of ecgonine or its esters; hence it appears certain that it existed as such in the seeds. Whether it is actually formed by the plant or is a product of chemical change after the ripening of the seed can only be disclosed by examination of fresh seeds. Those examined by us were of unknown age. It is worthy of note, however, that they were from widely separated sources, and that in both ecgonidine methyl ester was found to be the only alkaloid present in substantial amounts. If it were formed from an ecgonine ester, the change must have been sensibly complete before the seeds reached this Laboratory.

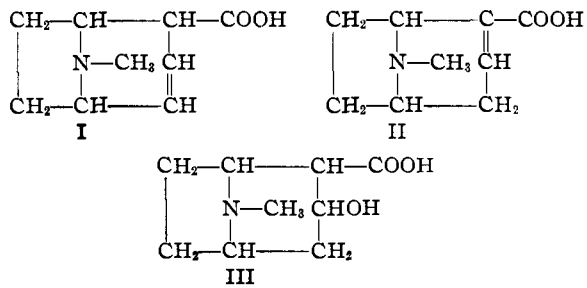
Ecgonidine (I or II), or anhydroecgonine, has been prepared by treatment of ecgonine (III) with dehydrating agents, such as phosphorus pentachloride[3] or phosphorus oxychloride.[4] The struc-

(1) Not copyrighted.

(2) Liebermann, *Ber.*, **40**, 3602 (1907).

(3) Merck, *ibid.*, **19**, 3002 (1886).

(4) Einhorn, *ibid.*, **20**, 1221 (1887).



I II III

ture is well established except for the position of the double bond, which is postulated as being in the 3,4-position (Formula I) by Willstätter,[5] and in the 2,3-position (Formula II) by Gadamer and John.[6] It is an amphoteric substance very soluble in water, from which it is not extracted by organic solvents. It is readily esterified and forms the usual complex salts, notably the periodide[4] which is characterized by its insolubility, unique crystal form, and convenient melting point.

Ecgonidine methyl ester is an oily hygroscopic base having an amine-like odor. It is readily hydrolyzed by acid to ecgonidine. The aurichloride (B·HCl·$AuCl_3$) is an excellent derivative for characterization, crystallizing in definite forms and melting at 152–153°.

The identity of the new alkaloid from the coca seeds was established by the relationships described below:

1. It was converted to ecgonidine methyl ester aurichloride, m. p. 152–153°.
2. It was converted to ecgonidine and thence to:
   - 2a. Ecgonidine periodide, m. p. 184–185°.
   - 2b. Ecgonidine methyl ester aurichloride, m. p. 152–153°.
   - 2c. Ecgonidine ethyl ester aurichloride, m. p. 123–124°.
3. Ecgonidine was prepared from cocaine by hydrolysis to ecgonine, followed by dehydration; and from it were prepared:
   - 3a. Ecgonidine periodide, m. p. 184–185°.
   - 3b. Ecgonidine methyl ester aurichloride, m. p. 152–153°.

The mixtures indicated below were prepared and found to melt at the same temperature as the individual substances:

1 and 2b, m. p. 152–153°
1 and 3b, m. p. 152–153°
2b and 3b, m. p. 152–153°
2a and 3a, m. p. 184–185°

Ecgonidine ethyl ester aurichloride prepared from the natural methyl ester (2c) melted at 123–124°. Liebermann[2] gives the melting point of this compound as 124°.

(5) Willstätter, *Ber.*, **31**, 2655 (1898).
(6) Gadamer and John, *Arch. Pharm.*, **259**, 227 (1921).

The refractive index of ecgonidine methyl ester extracted from the seeds was found to be the same as that of the alkaloid prepared from cocaine, *viz.*, 1.5023 at 20° (daylight).

Experimental Part

Extraction of the Alkaloid.—A 150-g. portion of coarsely ground seed was drenched with 25 cc. of concentrated ammonium hydroxide, and extracted with one 200-cc. portion, followed by three 100-cc. portions, of ether. The ether extracts were combined and concentrated to 30 cc., then extracted with three 15-cc. portions of normal hydrochloric acid solution. The acid solution, after being washed with ether to remove any oil carried over, was made alkaline with sodium carbonate and extracted with ether. Evaporation of the ether left a colorless, hygroscopic, sirupy liquid, with an odor of amines. It darkened on prolonged exposure to air and light. The yield was 0.03% in the case of the Java seed and 0.23% from Peruvian seed.

The aurichloride was formed by addition of 5% aqueous gold chloride to a solution of the alkaloid in 0.1 *N* hydrochloric acid. It precipitated in the form of characteristic yellow micro-crystals, m. p. 152–153°.[7] Neither crystal form nor melting point was changed on recrystallization from dilute acetone. *Anal.* Calcd. for $C_{10}H_{15}O_2N$·HCl·$AuCl_3$: Au, 37.84. Found: Au, 37.76, 37.85.

Preparation of Ecgonidine Periodide (a).—A solution of 0.2 g. of the alkaloid in 20 cc. of 5% aqueous hydrochloric acid was refluxed for six hours. The acid was neutralized with sodium carbonate and the solution evaporated to dryness. Ecgonidine hydrochloride was separated from the sodium chloride by solution in methanol. The periodide was precipitated from aqueous solution in the form of characteristic red-brown crystals by the addition of iodine–potassium iodide solution. Recrystallized from glacial acetic acid, they melted at 184–185°. Einhorn[4] gives 185°.

(b) Ecgonidine periodide was prepared from ecgonine according to the method of Einhorn[4]; m. p. 184–185°. A mixture of this product with that described under (a) above melted at 184–185°.

Ecgonidine methyl ester was prepared by refluxing 0.1 g. of ecgonidine from each of the sources described above with solutions of 2 cc. of concentrated sulfuric acid in 15 cc. of methanol. After diluting with water and making alkaline with sodium carbonate, the ester was extracted with ether. The aurichloride of each melted at 152–153°.

Mixtures of each of these aurichlorides with that of the natural alkaloid and with each other caused no depression in melting point.

The specific rotation of the alkaloid prepared from cocaine was −47.2° at 21° (*C* = 1.47 in alcohol).[8] Refractive index of the alkaloid prepared from both sources: 1.5023 at 20° (daylight).

Ecgonidine ethyl ester was prepared from ecgonidine (obtained from the natural alkaloid) in a similar fashion, substituting absolute ethanol for methanol; aurichloride, m. p. 123–124° (Liebermann[2] gives 124°).

Summary

An alkaloid, ecgonidine methyl ester, has been

(7) Fisher melting point apparatus.
(8) The rotation was determined using a bichromate filter according to the "Methods of Analysis" of the Assoc. Official Agr. Chem., Fifth Edition, 1940, p. 489.

isolated from the seeds of both *Erythroxylon coca* and *Erythroxylon novogranatense*. It is identical with ecgonidine methyl ester prepared from natural cocaine, and is subject to the provisions of the Harrison Narcotic Act.

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[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

Magnetic Measurements on Semiquinone Radicals in the Dissolved State

BY LEONOR MICHAELIS

Semiquinones arising in solutions of reversible dyestuffs on partial reduction have been identified in previous papers[1] as free radicals by using the method of slow reduction of the quinonoid form and observing the change, in time, of the magnetic susceptibility. In those previous papers the measurements were carried out with Will's apparatus, a modification of Quincke's method, based on the movement of the meniscus of the solution in the magnetic field. This method is very sensitive and often fulfills its purpose most satisfactorily. Difficulties are encountered with it in solutions of high viscosity; deep coloration of the solution is not favorable either. In the present paper another method, a modification of Gouy's, is used. Like Will's method, it is a differential one, and is essentially similar to that used by Freed and Kasper[2] and by Pauling and Coryell,[3] adapted to the particular purpose.

The "Isthmus electromagnet" (General Electric Company) with pole pieces 3 cm. in diameter, develops, at 10 amp. and pole gap of 1.35 cm. a field strength of 10,600 gauss, and under the conditions used in the following experiments the field strength is proportional to the current intensity at least up to 10 amp. The vessel suspended between the poles is a double vessel (1-cm. diameter, each compartment 10 cm. long), similar to that devised by Freed and Kasper, the dividing wall being located between the centers of the pole pieces. The solution to be measured is placed in the upper compartment, and some suitable liquid in the lower one, in order to compensate approximately the pull exerted on the upper half. In this way a differential method is established exhibiting high sensitivity. The compensating liquid is adjusted to the particular purpose. Often a 3% agar gel was used, which is free from the risk of rising air bubbles. In other cases, when working with a highly concentrated solution of sulfuric acid as a solvent, a similar solution of the acid without the dye was used as compensator.

The balance was a semi-micro magnetically-damped balance equipped with a scale of 200 divisions at the pointer which is read through a microscope, each division being equivalent approximately to a hundredth of a milligram. With such equipment the balance generally fulfills the task of a microbalance in a most convenient manner. During the experiment the weight of the vessel is balanced by counterweights so that the equilibrium position lies somewhere within the microscopic scale. All changes of susceptibility were measured by observing only the deflection on this scale arising from switching on the current abruptly with full strength, previously adjusted by a suitable resistance. Although under ordinary conditions the balance is very nearly critically damped, the deflection, after closing the current, exceeds the true equilibrium position a little. It is only the maximum deflection which is observed, and the calibration of the balance is made with respect to it. The resting position, before switching on the current, can be fixed to within ± 1 line of deflection, sometimes better, sometimes not quite so sharply, over a period of time sufficient to make the reading of a deflection which requires about fifteen seconds. Ample time should be given after breaking the current to reach again an equilibrium position as constant as possible, before again closing the current. The deflections are usually reproducible to ± 1, or ± 2 lines, and the amperage is chosen so as to reach a deflection of 20 to 120 lines, if possible. The readings were always recalculated for 10 amperes, on the assumption, ascertained by many preliminary measurements, that the deflection is strictly proportional to the square of the amperage under all conditions occurring, which shows that the time necessary to build up the magnetic field is negligibly small compared with the fifteen seconds needed to attain the maximum deflection. The deflections can be calibrated in terms of pull in milligrams, and even directly in terms of volume susceptibility, as follows. The lower compartment of the vessel is permanently filled with the compensator, say a 3% agar gel. The upper compartment is filled in one experiment with air, in another with water. To get the deflection into the range of the microscopic scale, for the vessel used in most of the experiments, 1.3 to 1.8 amp. are needed for air and 8 to 10 amp. for water. All values are recalculated for 10 amp. The algebraic difference of these two deflections corresponds, at 10 amp., to a change in volume susceptibility of 0.740×10^{-6} cgsm., of which $+0.020 \times 10^{-6}$ is the volume susceptibility of air, and -0.720×10^{-6} that of water. Herefrom each line of deflection can be directly calibrated in terms of susceptibility. Using the average value of ten successive readings, the calibration is reproducible to 1% and better, even over a period of weeks. With the vessel and pole distance used in most of

(1) L. Michaelis, G. F. Boeker, R. K. Reber, *et al.*, THIS JOURNAL, **60**, 202, 214, 1678 (1938).

(2) Simon Freed and Charles Kasper, *Phys. Rev.*, **36**, 1003 (1930).

(3) L. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci.*, **22**, 159 and 210 (1936); Chas. D. Coryell, Fred Stitt and Linus Pauling, THIS JOURNAL, **59**, 633 (1937).