siduronate) (XXVII), m.p. 189–190° (sealed capillary tube), $[\alpha]^{26}D$ −53° (*c* 1.27, methanol).

*Anal.* Calcd. for $C_9H_{13}O_6K$: C, 42.17; H, 5.11. Found: C, 41.95; H, 5.22.

A cold solution of 4.5 g. (17.6 mmoles) of the crude potassium salt XXVII in 40 ml. of water was rapidly acidified with 3 *N* hydrochloric acid and extracted with chloroform. The chloroform solution was washed with a saturated sodium chloride solution, dried over magnesium sulfate, and concentrated under reduced pressure yielding 3.1 g. (81%)[21] of methyl 2,3-isopropylidene-D-ribofuranosiduronic acid, m.p. 80–126°. A portion sublimed at 70–100° (1 mm.) gave an analytical sample, m.p. 134–135°, $[\alpha]^{25}D$ −70° (*c* 1.35, chloroform), $pH^{1/2}$ 3.7 (10% acetone–water solution).

*Anal.* Calcd. for $C_9H_{14}O_6$: C, 49.54; H, 6.47; $OCH_3$, 14.2; neut. equiv., 218. Found: C, 49.20; H, 6.30; $OCH_3$, 15.4; neut. equiv., 220.

**Methyl (Methyl 2,3-Isopropylidene-D-ribofuranosiduronate) (XXVI).**—A solution of 3.2 g. (14 mmoles) of methyl 2,3-isopropylidene-D-ribofuranosiduronic acid was suspended in 20 ml. of ether and treated with an excess of ethereal diazomethane. After 1 hour, the solution was concentrated giving 3.5 g. of oil. The oil was redissolved in ether and washed with dilute potassium bicarbonate solution. A residue of 3.2 g. of methyl (methyl 2,3-isopropylidene-D-ribofuranosiduronate) was obtained by concentrating the ethereal solution. A portion was evaporatively distilled at 60–80° (1 mm.) and yielded an analytical sample; $n^{25}D$ 1.4437, $[\alpha]^{25}D$ −74° (*c* 3.18, methanol).

*Anal.* Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95. Found: C, 51.33; H, 6.49.

**Methyl 5,5-Dimethyl-2,3-isopropylidene-D-ribofuranoside (XXVIII).**—A solution of 48 g. (0.344 mole, 21.5 ml.) of methyl iodide in 200 ml. of ether was added to 8.35 g. (0.344 mole) of magnesium ribbon in 40 ml. of ether over a period of about 2 hours. A solution of 20 g. (0.086 mole) of methyl (methyl 2,3-isopropylidene-D-ribofuranosiduronate) (XXVI) in 150 ml. of ether was added over a period of 1.5 hours. The mixture was refluxed 1 hour, cooled and poured onto a mixture of ice and 28.6 ml. of concentrated hydrochloric acid. The ether layer and 3 ether extracts were combined and washed with aqueous potassium bicarbonate. The ethereal solution was concentrated and the residue (19 g.) was distilled (b.p. 55° at 0.05 mm.) yielding 14.5 g. (73%) of methyl 5,5-dimethyl-2,3-isopropylidene-D-ribofuranoside, $n^{24}D$ 1.4442.

*Anal.* Calcd. for $C_{11}H_{20}O_5$: C, 56.88; H, 8.68. Found: C, 56.09; H, 8.30.

A 0.5-g. portion of the product was dissolved in 3 ml. of petroleum ether (b.p. 30–60°) and crystallized by cooling in a Dry Ice–acetone-bath. The product was recrystallized to yield 0.42 g. of methyl 5,5-dimethyl-2,3-isopropylidene-D-ribofuranoside, m.p. 35–36°, $[\alpha]^{24}D$ −63° (*c* 3.27, methanol).

*Anal.* Found: C, 56.97; H, 8.88.

(21) Although more than the theoretical amount of permanganate apparently was consumed during the reaction, considerable amounts of unreacted starting material were recovered in several reactions.

**5,5-Dimethyl-D-ribono-γ-lactone (XXIX).**—A solution of 3.72 g. (16 mmoles) of methyl 5,5-dimethyl-2,3-isopropylidene-D-ribofuranoside in 40 ml. of 0.1 *N* hydrochloric acid was refluxed until the rotation became constant (1.5 hours). The solution was cooled, neutralized with 4 g. of sodium bicarbonate and treated dropwise, while being stirred, with 0.8 ml. of bromine. After 1 hour a small amount of sodium sulfite was added and the solution was adjusted to *p*H 13 with 30% sodium hydroxide. After 1 hour, the solution was acidified (*p*H 2) with hydrochloric acid and kept at room temperature overnight. The rotation of the solution had become constant. The residue obtained after the solution was freeze-dried was continuously extracted (Soxhlet, 20 hours) with chloroform. Concentration of the solution yielded a crystalline residue. The residue was digested with 50 ml. of hot chloroform, cooled and filtered giving 1.7 g. (60%) of 5,5-dimethyl-D-ribono-γ-lactone, m.p. 130–132°. Three recrystallizations of a 100-mg. sample from methanol–ether yielded an analytical sample, m.p. 132–133°, $[\alpha]^{25}D$ +15° (*c*, 0.4, acetone), $\lambda_{max}^{Nujol}$ 5.71 μ, $\lambda_{max}^{CHCl_3}$ 5.63 μ (γ-lactone C=O).

*Anal.* Calcd. for $C_6H_{12}O_5$: C, 47.72; H, 6.87. Found: C, 47.52; H, 7.00.

**5,5-Dimethyl-2,3-isopropylidene-D-ribono-γ-lactone (XXX).**—A mixture of 500 mg. (2.84 mmoles) of 5,5-dimethyl-D-ribono-γ-lactone (XXIX), 50 ml. of acetone, 1 g. of anhydrous calcium chloride and a small amount of hydrogen chloride was stirred at room temperature for 16 hours. The mixture was filtered and the filtrate was stirred for 3 hours with about 0.5 g. of silver carbonate. The mixture was filtered and the filtrate was concentrated to dryness. The residue was dissolved in ether and filtered. The addition of petroleum ether (b.p. 30–60°) to the filtrate yielded 483 mg. of 5,5-dimethyl-2,3-isopropylidene-D-ribono-γ-lactone, m.p. 89–92°. A sample, recrystallized from benzene–hexane, melted at 90–92°, $[\alpha]^{25}D$ −55° (*c* 2, acetone), $\lambda_{max}^{CHCl_3}$ 5.63 μ (γ-lactone C=O).

*Anal.* Calcd. for $C_{10}H_{16}O_5$: C, 55.54; H, 7.46. Found: C, 55.80; H, 7.13.

**5,5-Dimethyl-4,5-di-O-methyl-2,3-isopropylidene-D-ribonic Acid (XXXII).**—Sodium 5,5-dimethyl-2,3-isopropylidene-D-ribonate (XXXI), prepared from 400 mg. (1.85 mmoles) of 5,5-dimethyl-2,3-isopropylidene-D-ribono-γ-lactone (XXX), was methylated in the manner described for sodium 5,5-dimethyl-2,3-isopropylidene-L-lyxonate (XXI). The crude methylation product (372 mg.) yielded 91 mg. of bicarbonate-soluble material and 234 mg. of bicarbonate insolubles. The infrared spectrum and melting point indicated that the bicarbonate-insoluble fraction was largely starting lactone XXX. The bicarbonate-soluble fraction was evaporatively distilled to yield 5,5-dimethyl-4,5-di-O-methyl-2,3-isopropylidene-D-ribonic acid, $[\alpha]^{25}D$ −24° (*c* 1.3, chloroform).

*Anal.* Calcd. for $C_{12}H_{22}O_6$: $OCH_3$, 23.66. Found: $OCH_3$, 21.32.

The infrared spectrum of the product showed a carboxyl band at 5.70 μ, but marked differences in the 8–10 μ region from the spectrum of the corresponding L-lyxonic acid XXIIa.

RAHWAY, N. J.

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[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES, DIVISION OF MERCK & CO., INC.]

# The Structure of Eulicin, a New Antifungal Agent

BY ROBERT E. HARMAN, EDWARD A. HAM, WILLIAM A. BOLHOFER AND NORMAN G. BRINK

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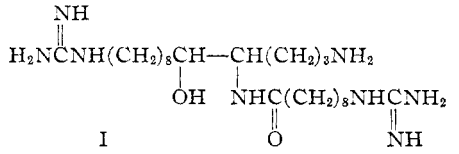
Degradative evidence is presented that the antifungal antibiotic eulicin ($C_{24}H_{52}O_2N_8$) possesses the structure I.

Recent communications[1,2] from these laboratories recorded the isolation of the antifungal agent eulicin, produced by a species of *Streptomyces*, the results of efficacy and toxicity studies and some preliminary chemical characterization. We wish to report here further work on the chemistry of eulicin which has culminated in the proposal of structure I for this antibiotic.

The only crystalline derivative of eulicin prepared in the previous study was the helianthate

(1) J. Charney, R. A. Macklowitz, F. J. McCarthy, G. A. Rutkowski, A. A. Tytell and W. P. Fisher, "Antibiotics Annual," Medical Encylopedia, Inc., New York, N. Y., 1955–1956, pp. 228–230.

(2) M. K. West, W. F. Verway and A. K. Miller, *ibid.*, 1955–1956, p. 231.



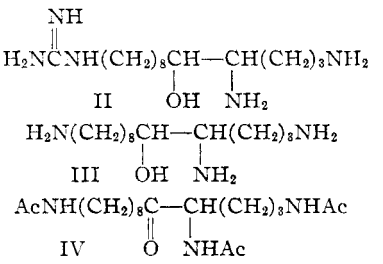
I

salt. Analytical data on this substance and on the amorphous hydrochloride indicated an equivalent weight of about 165. The strong basicity of eulicin and the positive Sakaguchi reaction suggested guanidino functionality. Analytical data were indeterminate because of persistent inorganic impurities, but indicated absence in the base of elements other than carbon, hydrogen, oxygen and nitrogen. Subsequent attempts to obtain other crystalline salts have been unsuccessful; the free base, prepared *via* IRA-400 on the hydroxyl cycle, was a hygroscopic gum which underwent decomposition in a few hours with release of ammonia. Analytical data on derivatives of the intact antibiotic have not been sufficiently meaningful to permit assignment of an empirical formula. Nevertheless the results of solubility analyses, paper chromatography and attempted fractionation on IRC-50 resin, as well as the consistent reproducibility of the chemical degradations, indicated that all the eulicin samples under study were essentially homogeneous.

Alkaline hydrolysis of eulicin gave ammonia in somewhat more than the expected amount, together with a mixture of non-volatile, ninhydrin-positive bases. A negative Sakaguchi test showed the complete loss of guanidino groups. A small amount of 9-aminononanoic acid, which was identified by direct comparison with an authentic sample,[3] was isolated from the reaction mixture.

Acid hydrolysis of eulicin yielded an amorphous base, designated eulicinine (II), and a crystalline guanidino acid. The latter was identified as 9-guanidinononanoic acid by analysis, infrared absorption spectrum characteristics, and conversion by alkaline hydrolysis to 9-aminononanoic acid. Like eulicin, eulicinine failed to form any crystalline derivatives of low molecular weight, and hence meaningful physical data again are lacking. Eulicinine estimated to be of over 90% purity was obtained by partition chromatography. Analytical data suggested that it formed a trihelianthate. The compound could be detected on papergrams by both ninhydrin and Sakaguchi reagents, and so was presumed to be an amino guanidine. Isolation of eulicinine and the guanidino acid from the acid hydrolysis of eulicin confirmed the assignment to eulicin of two guanidine residues and suggested the presence of an amide or amidine group in the antibiotic. No ammonia was liberated during the acid hydrolysis, thus defining the site of cleavage as an amide and not an amidine group.

When eulicinine was hydrolyzed with alkali, ammonia was obtained, again in somewhat more than the expected amount. The aqueous hydrolysate gave in moderate yield eulamine (III), an odorous, low-melting, hygroscopic solid which was obtained crystalline only by sublimation. Treatment with acetic anhydride in ethyl acetate solution converted eulamine in high yield to a crystalline derivative. Analytical and infrared data showed this to be a triacetylamino alcohol. The derivative was further characterized by oxidation to the triacetylamino ketone IV, and by further



II

III

IV

acetylation in pyridine solution to an amorphous tetraacetyl derivative. Eulamine itself was assigned the composition $C_{13}H_{31}ON_3$ on the basis of analyses on the triacetyl derivative and potentiometric titration of the base.

Oxidation of eulamine with nitric acid afforded a mixture of dibasic acids. These were separated by partition chromatography in a chloroform–water–1-butanol system and identified as suberic, adipic, glutaric and succinic acids. In experiments with model compounds, suberic acid was recovered unchanged after exposure to the same vigorous conditions of oxidation, but cyclohexylamine and 1,10-diaminodecane gave mixtures of dibasic acids. In a Kuhn–Roth oxidation, eulamine yielded no acetic acid. The results of these oxidative studies demonstrated that the eulamine skeleton contained an unbranched, unsubstituted chain of at least eight carbon atoms.

The $\alpha$-amino alcohol group and the carbon skeleton of eulamine were revealed by periodate oxidation studies. The reaction in acidic medium was unsatisfactory: slow oxidation of eulamine was observed, with no clean break in the time–consumption curve, and iodate also was consumed. However, in basic solution extremely rapid uptake of one equivalent of periodate occurred. This behavior is characteristic of that reported for $\alpha$-amino alcohols.[4] Ammonia was detected among the volatile products of the reaction, but neither formaldehyde nor formic acid could be found. The reaction mixture was shown by paper chromatography to contain several ninhydrin-positive products. It initially gave positive tests for aldehydic components with 2,4-dinitrophenylhydrazine and with fuchsin reagent, but these tests were negative after the solution had been either lyophilized or stored for several hours at room temperature.

In an attempt to stabilize the periodate reaction products sufficiently to permit identification, the reaction mixture was oxidized directly with permanganate. 9-Aminononanoic acid and a trace of impure material which had the same $R_f$ value on papergrams as $\gamma$-aminobutyric acid were isolated. This suggested that the initial periodate products were the corresponding $\omega$-amino aldehydes. This

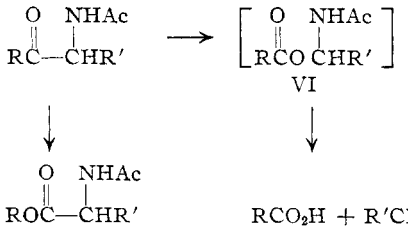
(3) T. Gäumann and Hs. H. Günthard, *Helv. Chim. Acta*, **35**, 53 (1952). We are indebted to Dr. T. Gäumann, Eidg. Tech. Hochschule, Zurich, Switzerland, who kindly supplied us with samples of the amino acid and its hydrochloride for the comparison.

(4) D. D. Van Slyke, A. Hiller and D. A. MacFadyen, *J. Biol. Chem.*, **141**, 681 (1941).

was confirmed by a series of reactions in which eulamine was oxidized with periodate, the reaction mixture acetylated directly, and the stabilized ω-acetylamino aldehydes converted to 2,4-dinitrophenylhydrazones and separated by adsorption chromatography on silica gel. The 4-acetylaminobutanal derivative was identified by comparison with an authentic sample and that of 9-acetylaminononanal by microanalysis. Eulamine was thus assigned a straight chain 13-carbon α,ω-diamine structure with α-amino alcohol functionality on carbon atoms 4 and 5. It was not possible, of course, to distinguish between $C_4$ and $C_5$ with respect to the locations of the amino and hydroxyl groups of the α-amino alcohol.

As a first approach to a degradation of eulamine which might reveal the relative positions of these groups, we attempted to prepare a derivative in which the α-amino alcohol was converted to an α-amino ketone, since periodate or tetraacetate cleavage of such a compound would be expected to yield the desired information. Unfortunately, no characterizable amino ketone could be obtained either directly from eulamine or by hydrolysis of the previously described triacetylamino ketone.

The structure of eulamine finally was elucidated by a study of the oxidation of triacetylamino ketone IV with peroxytrifluoroacetic acid, a reagent known to convert ketones to esters in high yield.[5] In the present case it was anticipated that either an ester (V) of an α-acetylamino acid or an acylated aldehyde–ammonia adduct (VI) would be formed. The products obtained were those expected from hydrolysis of the unstable aldehyde–ammonia adduct, 9-acetylaminononanoic acid and 4-acetylaminobutanal, the latter isolated as the 2,4-dinitrophenylhydrazone. No other acidic or aldehydic




reaction products were present in identifiable amounts. Thus the nitrogen function of the α-amino alcohol moiety of eulamine was demonstrated to be on carbon atom 4, and eulamine was assigned structure III.

Since there appears to be no literature precedent for this application of peroxytrifluoroacetic acid oxidation, a model experiment was done. 3-Acetylamino-5-methylhexanone-2[6] was oxidized with the peracid and found to yield isovaleraldehyde. Thus in this case also oxygen was introduced into the carbon chain between the carbonyl carbon atom and the adjacent carbon atom bearing the acetylamino group.

With the structure of eulamine established as represented in formula III, the locations of the 9-guanidinononanoyl residue and the second guanidino group in the parent eulicin remained to be determined. Eulicinine was cleaved readily by periodate with the formation, after further oxidation with permanganate, of 9-guanidinononanoic acid. This result is consistent only with structure II. Finally, it was observed that eulicin itself did not react with periodic acid. Hence the guanidinoacyl residue was shown to be on the $C_4$-amino group, and eulicin was assigned structure I.

The stereochemistry of the weakly dextrorotatory substances eulicin, eulicinine and eulamine, each having two asymmetric carbon atoms, remains to be established.

**Acknowledgment.**—Dr. R. G. Chase and his associates prepared the crude eulicin used in this study. The authors are indebted to Mr. Robert Walker for the infrared data, to Mr. R. N. Boos and his associates for the microanalyses, and to Mr. J. Wittick for the potentiometric titrations.

## Experimental[7]

**Eulicin Hydrochloride.**—A solution of 1 g. of crude eulicin helianthate in 25 ml. of hot methanol was cooled to incipient crystallization. Concd. hydrochloric acid (0.5 ml.) was added, the mixture cooled in ice for an hour and the dye filtered. The last traces of methyl orange were removed from the filtrate by passage through a 0.5-g. Norite column. Supercel (3 g.) was suspended in the colorless solution, 35 ml. of acetone added with stirring and then 200 ml. of ether. The solid was filtered, washed with ether, and finally extracted on the funnel with a total of 20 ml. of water. Lyophilization gave 385 mg. (92%) of eulicin trihydrochloride ($R_f$ 0.7, strong positive Sakaguchi reaction, weak ninhydrin) as a hygroscopic white powder. All samples studied had poorly defined infrared spectra with broad absorption near 3 μ and at 6–6.5 μ.

*Anal.* Calcd. for $C_{24}H_{52}O_2N_8 \cdot 3HCl$: Cl, 17.91. Found: Cl, 17.55.

**Eulicin Acetate.**—A solution of 400 mg. (0.29 millimole) of the helianthate in about 15 ml. of warm methanol was put on a 20-ml. column of IRA-400 resin on the acetate cycle. The Sakaguchi-positive effluent was taken to dryness to yield 180 mg. (104%) of amorphous hygroscopic acetate, $R_f$ 0.7. The infrared spectrum was poorly defined and like that of the hydrochloride. Analytical data were not entirely satisfactory but suggested that the salt was a diacetate.

*Anal.* Calcd. for $C_{24}H_{52}O_2N_8 \cdot 2HOAc$: N, 18.53; HOAc, 19.8. Found: N, 19.85; HOAc, 16.6, 17.8.

**Eulicin Helianthate.**—Before conversion to the helianthate, eulicin hydrochloride was dissolved in 10 parts of water, strong sodium hydroxide solution added to *p*H about 12, and any precipitated inorganic hydroxides centrifuged and discarded. The filtrate was brought to *p*H 8–9 with hydrochloric acid and a warm solution of 2.0 g. of methyl orange in 100 ml. of water was added per gram of eulicin hydrochloride. The flocculent, slightly gummy salt was centrifuged, the supernatant discarded and the salt washed with water. It was then crystallized from 80% methanol–water, 75% methanol–ethyl acetate and finally methanol. The best samples melted with decomposition at 154–156°, with sintering at 142°.

*Anal.* Calcd. for $C_{24}H_{52}O_2N_8 \cdot 3C_{14}H_{15}O_3N_3S$: C, 56.59; H, 6.98; N, 17.00; S, 6.85; methyl orange·HCl, 73.2. Found: (average of 4) C, 55.98; H, 6.77; N, 18.60; S, 7.48; C–$CH_3$, 0.0; methyl orange·HCl, 70.8.

**Alkaline Hydrolysis of Eulicin.**—A solution of 120 mg. (0.20 millimole) of eulicin hydrochloride in 12 ml. of 0.5 *N*

(5) W. D. Emmons and G. B. Lucas, THIS JOURNAL, **77**, 2287 (1955).

(6) H. D. Dakin and R. West, *J. Biol. Chem.*, **78**, 91 (1928).

(7) Melting points were determined on a micro hot-stage. Infrared spectra were obtained on a Baird Associates infrared recording spectrophotometer model B. Disk paper chromatography was carried out in the system 1-butanol–water–acetic acid (4:5:1) unless otherwise specified. Partition columns were prepared by slurrying Supercel in excess of upper phase from equilibration of equal volumes of 1-butanol and 1% acetic acid, and then shaking vigorously with 0.6 ml. of lower phase per gram of Supercel. Columns were packed by gravity alone. Substrate was applied to the columns in solution in a small volume of upper phase.

barium hydroxide was heated under reflux. A stream of nitrogen was passed through the solution and the liberated volatile base collected in standard acid. Titration indicated a total of 0.96 millimole (4.8 moles per mole, or 120% of theory) of base released during 20 hours. The volatile base was shown to be ammonia by isolation as ammonium chloride, identified by infrared absorption spectrum, and by subsequent conversion to 2,4-dinitroaniline by reaction with 2,4-dinitrofluorobenzene.

The aqueous hydrolysate was filtered, acidified to *p*H 5 with sulfuric acid, the precipitated barium sulfate removed and the filtrate lyophilized. The product, 105 mg. of nearly colorless gum, gave a negative Sakaguchi reaction. Paper chromatography showed it to be a mixture of five ninhydrin-positive substances with $R_f$ values from 0.1 to 0.78. Partition chromatography of 520 mg. of the gum on a column[7] prepared from 90 g. of Supercel afforded about 50 mg. of a crystalline amino acid ($R_f$ 0.78) in early effluents; no other well-defined products were isolated. The acid, after recrystallization from methanol, melted at 189–190° and was identical with an authentic sample of 9-aminononanoic acid[3] by mixed melting point and infrared comparisons.

*Anal.* Calcd. for $C_9H_{19}O_2N$: C, 62.39; H, 11.05; N, 8.09; equiv. wt., 173. Found: C, 62.56; H, 10.96; N, 8.68; equiv. wt. (potentiometric titration), 173.

The acetyl derivative of 9-aminononanoic acid was prepared by heating on the steam-cone for two hours a solution of 43 mg. of the acid in 1 ml. of acetic acid containing 31 mg. (1.2 equivalents) of acetic anhydride. Solvents were then removed *in vacuo* and the residue was crystallized from ethyl acetate. The acetylamino acid melted at 70–71° and had $\lambda_{max}^{Nujol}$ 3.05, 5.87, 5.92, 6.09 and 6.38 $\mu$.

*Anal.* Calcd. for $C_{11}H_{21}O_3N$: $CH_3CO$, 19.98. Found: $CH_3CO$, 20.37.

**Acid Hydrolysis of Eulicin.**—Preliminary investigations in which the results were followed by paper chromatography[8] showed that eulicin was cleaved slowly at 25° and more rapidly at 100° by 4 *N* hydrochloric acid to yield two new substituted guanidines (Sakaguchi test) of $R_f$ values 0.5 and 0.85. In a preparative experiment, 2.79 g. of eulicin acetate was dissolved in 25 ml. of 4 *N* acid and heated on the steam-cone for four hours. The hot solution was decolorized with Norite and cooled in ice for one hour. The white crystalline 9-guanidinononanoic acid hydrochloride (0.93 g., $R_f$ 0.85) was then collected and the filtrate reserved for isolation of eulicinine.

Recrystallized from 4 *N* hydrochloric acid, the guanidino acid hydrochloride melted at 165–166° dec.; its infrared spectrum in Nujol was characterized by bands at 2.96, 3.18, 5.82, 6.0 (broad) and 6.16 $\mu$.

*Anal.* Calcd. for $C_{10}H_{22}O_2N_3Cl$: N, 16.69; Cl, 14.08. Found: N, 16.73; Cl, 13.91.

Alkaline hydrolysis of the guanidino acid for 24 hours in refluxing barium hydroxide solution followed by removal of barium with sulfuric acid and then elimination of sulfate ion on a column of IR-45 (hydroxide cycle) gave 9-aminononanoic acid, m.p. 189–190°, identified by mixed melting point and infrared comparison with an authentic sample.

The eulicinine-containing filtrate from the guanidino acid hydrochloride was evaporated to dryness, freed of chloride ion on a 50-ml. IRA-400 column (acetate cycle) and fractionated on a partition column[7] prepared from 200 g. of Supercel. A series of 135-ml. fractions was collected. The first three fractions contained a further 155 mg. of 9-guanidinononanoic acid. These were followed by five nearly solute-free fractions. After this, ten 270-ml. fractions were collected containing 1.70 g. of crude eulicinine acetate (II). Paper chromatograms showed a single spot, both ninhydrin- and Sakaguchi-positive, at $R_f$ 0.5.

Eulicinine helianthate (m.p. 155–158° dec.) was prepared as described above for eulicin helianthate.

*Anal.* Calcd. for $C_{14}H_{33}ON_5 \cdot 3C_{14}H_{15}O_4N_3S$: methyl orange·HCl, 85.2. Found: methyl orange·HCl, 82.0.

A non-crystalline acetate salt, $[\alpha]^{25}D$ +3° (*c* 0.77 in water), was prepared from the helianthate by use of IRA-400 on the acetate cycle. Unexpectedly, analysis showed that the salt had the composition of a diacetate.

*Anal.* Calcd. for $C_{14}H_{33}ON_5 \cdot HOAc$: N, 20.2; HOAc, 17.3. For $C_{14}H_{33}ON_5 \cdot 2HOAc$: N, 17.2; HOAc, 29.5. Found: N, 20.8; HOAc, 28.8.

**Eulamine (III).**—A 416-mg. quantity (1.2 millimoles) of eulicinine acetate was hydrolyzed by heating under reflux overnight in 40 ml. of 0.5 *N* barium hydroxide solution. A volatile base, identified as ammonia by the infrared spectrum of its hydrochloride, was liberated. The yield was 2.68 millimoles, or 116% of theory. Crude eulamine (230 mg.) was isolated from the aqueous hydrolysate by removal of barium with sulfuric acid, lyophilization of the filtrate and extraction of the residue with 1-butanol. The product had an $R_f$ value of 0.45, ninhydrin test positive, Sakaguchi test negative. Sublimation at 150° and 0.1 mm. gave 160 mg. of crystalline eulamine, m.p. 57° in the original evacuated sublimation tube. The material melted below 40° after short exposure to air. The infrared spectrum of eulamine was poorly defined; maxima were observed at about 3 $\mu$ and in the 6.0–6.4-$\mu$ region. Eulamine had $[\alpha]^{25}D$ +6° (*c* 1.28 in water).

*Anal.* Calcd. for $C_{13}H_{31}ON_3$: N, 17.1; equiv. wt., 81.8. Found: N, 17.8, 16.6; equiv. wt. (formol titration), 83; C-$CH_3$, none.

**Tri-N-acetyleulamine.**—One hundred milligrams of eulamine was suspended in 3 ml. of ethyl acetate, 0.3 ml. of acetic anhydride added and the mixture boiled for about a minute, during which time complete solution of the material occurred. Crystallization of the product took place in almost quantitative yield when the solution was cooled. The tri-N-acetyleulamine, recrystallized from methanol–ethyl acetate, melted at 140–141° and had $[\alpha]^{25}D$ +20.6° (*c* 2.96 in methanol) and +14° (*c* 1.55 in water). The infrared spectrum in Nujol was characterized by bands at 2.92, 3.02, 6.1 (broad), 6.52, 7.70, 8.32, 8.60 and 8.84 $\mu$.

*Anal.* Calcd. for $C_{13}H_{28}ON_3(CH_3CO)_3$: C, 61.42; H, 10.04; N, 11.31; $CH_3CO$, 34.76. Found: C, 61.54; H, 9.83; N, 10.85; $CH_3CO$, 35.4.

A 7-mg. sample of tri-N-acetyleulamine was dissolved in 1 ml. of pyridine, 0.5 ml. of acetic anhydride added and the mixture heated for 10 minutes on the steam-cone. Evaporation of the solvents *in vacuo* gave 9 mg. of a colorless gum that could not be crystallized. Its infrared spectrum was essentially the same as that of the triacetyl derivative, except for the appearance of a new band at 5.78 $\mu$ (ester).

**Chromic Acid Oxidation of Triacetyleulamine.**—A slurry of 200 mg. of chromium trioxide (2.0 millimoles) in 2 ml. of dry pyridine was added to a solution of 175 mg. (0.47 millimole) of triacetyleulamine in 8 ml. of pyridine. After 16 hours at 25°, the dark suspension was taken to dryness *in vacuo* at 25° and the residue extracted with ethyl acetate to yield 165 mg. (93%) of crude ketone as a nearly colorless semi-solid. Crystallization from methanol–ethyl acetate yielded 123 mg. of triacetylamino ketone IV as initially gelatinous globules which slowly became granular and then melted at 146–148° after sintering at 135°; $\lambda_{max}^{Nujol}$ 3.03, 5.84, 6.05 (broad), 6.52, 7.70, 8.33, 9.50 and 9.90 $\mu$.

**Nitric Acid Oxidation of Eulamine.**—A 178-mg. sample of eulamine was dissolved in 2 ml. of ice-cold concd. nitric acid. The solution was then allowed to come to room temperature and finally heated at 100° for 24 hours. Removal of the solvents *in vacuo* left 110 mg. of partly crystalline residue. Papergram analysis in the system 4% 1-butanol in chloroform–$H_2O$ (1:1) showed the presence of acids of $R_f$ values 0.10, 0.27, 0.44, 0.65 and 0.86, which corresponded, respectively, to succinic (two slowest spots), adipic, glutaric and suberic acids. These acids were obtained pure and identified by melting point and infrared comparisons with known samples after separation of the crude mixture by partition chromatography[9] in the system chloroform–butanol–water.

Sebacic acid was recovered quantitatively, unchanged, from similar vigorous nitric acid oxidation. Papergram analysis of products obtained likewise from 1,10-diaminodecane and cyclohexylamine showed that each of these,

(8) In order to avoid difficulties encountered in the paper chromatography of hydrochloric acid hydrolysates, apparently caused by the presence of different ionic species of the same product, the papergram studies were carried out on samples that had been freed of chloride ion by treatment with IRA-400 resin on the acetate cycle.

(9) The method of C. S. Marvel and R. D. Rands, Jr., THIS JOURNAL, **72**, 2642 (1950), was used except that Supercel was substituted for silicic acid.

like eulamine, was converted into a series of dicarboxylic acids.

**Periodate Oxidation of Eulamine. A.**—To a solution of 11 mg. (0.045 millimole) of eulamine in 9 ml. of phosphate buffer, *p*H 7.7, was added 1.00 ml. of 0.0947 *M* sodium periodate solution. A pungent odor (γ-aminobutanal) developed at once upon addition of the periodate. Consumption of oxidant was 0.95 equivalent in three minutes, 1.65 equivalents in 28 minutes and 2.05 equivalents in 130 minutes. Papergram analysis of the products after 5 minutes reaction time showed ninhydrin-positive substances with $R_f$ values of 0.17, 0.26, 0.52, 0.62, 0.73 and 0.85.

**B.**—A 56-mg. quantity of eulamine was oxidized by excess periodate in a solution which was about half-saturated with potassium carbonate. The volatile bases produced were carried by a stream of nitrogen into 3 ml. of dilute hydrochloric acid. Lyophilization of the acid gave a partly crystalline residue, a mixture of at least four ninhydrin-positive substances of $R_f$ values 0.05, 0.17, 0.30 and 0.44. Ammonium chloride (8 mg., $R_f$ 0.30) was isolated from the mixture by fractional precipitation from methanol solution by ethyl acetate, and was identified by its infrared spectrum.

**C.**—A solution of 120 mg. of eulamine (0.49 millimole) in 5 ml. of water was treated with 10.2 ml. of 0.1 *M* periodic acid solution. Within a minute a solution of 103 mg. of potassium permanganate in 10 ml. of water was added and rapidly consumed. A further 52 mg. of permanganate was added and consumed within 20 minutes. The precipitated manganese dioxide was removed and the filtrate lyophilized and extracted with methanol to yield 131 mg. of colorless gum. Papergram analysis showed ninhydrin-reacting components of $R_f$ values 0.31, 0.40, 0.49, 0.62, 0.68 and 0.78. By comparison, γ-aminobutyric and 9-aminononanoic acids had $R_f$ values of 0.48 and 0.79, respectively.

The mixture of products was partitioned between 1-butanol and 1% acetic acid and the butanol-soluble material then distributed between ethyl acetate and water. The water-soluble fraction was passed over a 5-ml. column of IR-45 (hydroxide cycle). Elution of the column with water gave 29 mg. of 9-aminononanoic acid, identified by comparison with authentic material. The aqueous fraction from the butanol–acetic acid partition of the crude oxidation product yielded a small amount of amorphous material of $R_f$ 0.48, probably largely γ-aminobutyric acid.

**D.**—Six milliliters of 0.1 *M* periodic acid was added rapidly and with stirring to a solution of 125 mg. (0.48 millimole) of eulamine in 1 ml. of water. Acetic anhydride (300 mg., 3 millimoles) was added at once and a few drops of dilute alkali introduced to bring the solution near neutrality. After 30 minutes the reaction mixture was passed over a 10-ml. column of IRA-400 resin (hydroxide cycle) to free it of iodate and periodate, and then treated with excess 2,4-dinitrophenylhydrazine. The resulting suspension was extracted repeatedly with benzene.

Papergram studies in the solvent system hexane–50% aqueous methanol (1:1) indicated three components: excess reagent at $R_f$ 0.58, and derivatives at $R_f$ 0.80 and 0.92. These were partially separated during the successive benzene extractions and the derivatives finally obtained pure by adsorption chromatography of appropriate fractions on silica gel. Excess reagent was eluted by chloroform, ethyl acetate brought a clean band of the substance of $R_f$ 0.92 from the column, and methanol eluted the material of $R_f$ 0.80. This separation procedure yielded 28 mg. of 4-acetylaminobutanal 2,4-dinitrophenylhydrazone, $R_f$ 0.80, m.p. 175–177°, identified by direct comparison (mixed m.p., infrared spectrum) with an authentic sample (see below), plus an additional 29 mg. of somewhat impure derivative. The substance of $R_f$ 0.92, 9-acetylaminononanal 2,4-dinitrophenylhydrazone, 33 mg., melted at 146–148°; its infrared spectrum in Nujol had maxima at 3.02, 5.35, 6.12, 6.55 and 7.0 μ.

*Anal.* Calcd. for $C_{17}H_{25}O_5N_5$: C, 53.81; H, 6.64; N, 18.46. Found: C, 53.80; H, 6.71; N, 18.53.

**Peroxytrifluoroacetic Acid Oxidation of Triacetylamino Ketone IV.**—A solution of peroxytrifluoroacetic acid was prepared by dropwise addition with stirring of 0.25 ml. of trifluoroacetic anhydride to an ice-cold mixture of 0.04 ml. of 90% hydrogen peroxide and 0.25 ml. of ethylene dichloride. Ninety microliters of the homogeneous solution, containing 0.24 millimole of reagent, was added to a slurry of 45 mg. (0.12 millimole) of the acetylamino ketone IV and 80 mg. of disodium hydrogen phosphate in 0.3 ml. of ethylene dichloride. After one hour at room temperature, solvents were removed *in vacuo*, the residual gum was taken up in 4 ml. of water, and 1.2 ml. of 2,4-dinitrophenylhydrazine reagent[10] (0.15 millimole) added. After several hours the crystalline product which had separated was collected. An ethyl acetate solution of the crude derivative (22 mg.) was put on a 3-ml. column of silica gel. The column was washed with ethyl acetate and then eluted with methanol. The methanolic eluate yielded 18.5 mg. of yellow crystalline 4-acetylaminobutanal 2,4-dinitrophenylhydrazone, m.p. 173–175°, identified by comparison with an authentic sample.

The aqueous acid filtrate from preparation of the phenylhydrazone was extracted with ethyl acetate and the ethyl acetate solution then extracted with saturated aqueous sodium bicarbonate solution. Acidification of the bicarbonate solution and extraction with ethyl acetate afforded 20 mg. of brown partly crystalline residue. Crystallization from 0.2 ml. of ethyl acetate gave 9-acetylaminononanoic acid, m.p. 67–71°. It was identified by infrared comparison with a known specimen.

**Peroxytrifluoroacetic Acid Oxidation of 3-Acetylamino-5-methylhexanone-2.**—Crude 3-acetylamino-5-methylhexanone-2 was synthesized[6] from leucine and characterized by preparation of its phenylhydrazone,[6] m.p. 110–113°, and 2,4-dinitrophenylhydrazone, m.p. 177–178°.

*Anal.* Calcd. for $C_{15}H_{21}O_5N_5$: N, 19.93; $CH_3CO$, 12.25. Found: N, 20.08; $CH_3CO$, 11.76.

A 157-mg. (0.92 millimole) sample of the α-acetylamino ketone was oxidized as described above with a 1.5-fold excess of peroxytrifluoroacetic acid and the product treated with the dinitrophenylhydrazine reagent. The resulting crude derivative was chromatographically purified on a silica gel column to yield 78 mg. (30%) of isovaleraldehyde 2,4-dinitrophenylhydrazone, m.p. 123–124°. Its identity was established by melting point and infrared comparisons with a specimen of the authentic derivative.[11]

**4-Aminobutanal 2,4-dinitrophenylhydrazone.**—A solution of 400 mg. of potassium iodide and 10 g. (0.15 mole) of potassium cyanide in 20 ml. of water was added to 16.6 g. (0.1 mole) of 3-chloropropanal diethylacetal[12] in 100 ml. of ethanol. After 16 hours at reflux, the bulk of the solvent was removed by distillation, the residue mixed with 20 ml. of water and the crude product (15 g.) isolated by extraction with benzene. Fractionation at 10 mm. pressure in a 2-foot Podbielniak-type column gave a small fore-run of unchanged chloroacetal and 9.5 g. (60%) of cyanoacetal boiling at 97°. A 1.58-g. (10-millimole) sample was hydrogenated at 2100 p.s.i. and 100° in the presence of 0.5 teaspoon of Raney nickel, 10 ml. of liquid ammonia and 3 ml. of absolute alcohol. The solvent was then removed, the residue taken up in 10 ml. of benzene and 1.5 ml. (0.15 millimole) of acetic anhydride added. The solution warmed spontaneously and then was boiled briefly. Solvent was removed *in vacuo* from 1.2 ml. (1.0 millimole) of this solution of the acetylaminoacetal, the residual gum taken up in 2 ml. of methanol and 5 ml. of water, and 200 mg. of 2,4-dinitrophenylhydrazine[10] added. Filtration after 8 hours and crystallization twice from aqueous methanol gave 220 mg. (71%) of 4-acetylaminobutanal 2,4-dinitrophenylhydrazone of m.p. 176–177°; $\lambda_{max}^{Nujol}$ 3.02, 4.35, 6.10, 6.19, 6.32 and 6.55 μ.

*Anal.* Calcd. for $C_{12}H_{15}O_5N_5$: C, 46.59; H, 4.88; N, 22.64. Found: C, 47.11; H, 5.00; N, 23.19.

**Periodate Oxidation of Eulicinine.**—A 25-mg. (0.072 millimole) sample of eulicinine acetate, when treated with excess periodate at *p*H 7.7 as described previously for the oxidation of eulicin, consumed 0.64 equivalent of reagent in 3 minutes and 0.75 equivalent in 113 minutes. The odor of the $C_4$-aldehyde was noticeable during the reaction. Paper chromatographic analysis of the products after the reaction had proceeded for 5 minutes showed ninhydrin-reactive material of $R_f$ values 0.28 and 0.84, the latter being also Sakaguchi positive.

In a preparative experiment, a solution of 149 mg. (0.32 millimole) of eulicinine acetate in 10 ml. of water was

(10) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 171.

(11) R. L. Shriner and R. C. Fuson, *ibid.*, p. 229.

(12) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 137.

treated with 3.3 ml. of 0.1 *M* periodic acid solution, and drops of bicarbonate solution were then added to restore the faint basicity of the mixture. After 10 minutes the solution was brought to *p*H 4 with acetic acid and 67 mg. of potassium permanganate in 6.7 ml. of water added. The solution was decolorized with solid sodium bisulfite after one-half hour, the manganese dioxide centrifuged and the supernatant lyophilized. The residual solid was taken up in 1 ml. of lower phase from equilibration of equal volumes of 1-butanol and 1% acetic acid and extracted with six 1-ml. portions of upper phase. The latter yielded 67 mg. of colorless gum which was put on a solvent-partition column[7] prepared from 1 g. of Supercel. Early fractions gave crystalline material which was combined and recrystallized from 1 ml. of 8 *N* hydrochloric acid. A yield of 24 mg. of 9-guanidinonanoic acid hydrochloride, m.p. 164–165° dec., was obtained. The material was identified by melting point and infrared comparisons with an authentic sample.

**Periodate Treatment of Eulicin.**—Under the conditions described above for the oxidation of eulamine and eulicinine with periodate, eulicin was resistant to attack. A 30-mg. (0.05 millimole) sample of the acetate salt, treated with two equivalents of periodate at *p*H 7.7, consumed 0.11 equivalent in 3 minutes and 0.38 equivalent in 126 minutes. Eulicin hydrochloride behaved similarly. No odor developed during the oxidations. At the end of 5 minutes, papergram analysis of the reaction mixture showed only unchanged eulicin at $R_f$ 0.70.

RAHWAY, N. J.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF BRANDEIS UNIVERSITY]

# Aspidospermine. II. Nuclear Magnetic Resonance Spectra and Classical Degradations[1]

BY HAROLD CONROY, PETER R. BROOK, MAHENDRA K. ROUT AND NORMAN SILVERMAN

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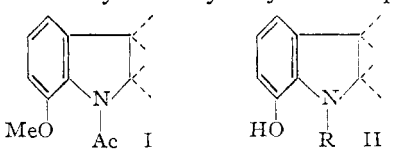
Nuclear magnetic resonance spectra of the alkaloid aspidospermine and a number of its degradation products are recorded and their structural implications discussed. The Hofmann, Emde and von Braun degradations have been carried out. An N-methyl group, thought previously to be present on the basis of n.m.r. spectra and direct Herzig–Meyer determinations, has been excluded by these classical methods and by the fact that inactive aspidospermine can be recovered by decomposition of its radioactive N-methyl-C-14 methiodide. Evidence obtained leads to the formulation of the environment at $N_b$.

Aspidospermine, $C_{22}H_{30}O_2N_2$, one of the alkaloids of *Aspidosperma quebracho blanco* and of *Vallesia glabra*, was first studied from a chemical viewpoint by Ewins[2]; he found it to contain a methoxylated aromatic ring, an acetamido grouping and a tertiary, basic nitrogen atom. Spectral comparisons of aspidospermine and derivatives with model substances allowed Openshaw,[3] Witkop[4] and their coworkers to conclude that a 7-methoxy-1-acetylindoline system (I) is present. Deacetylaspidospermine, obtained by acid hydrolysis of aspidospermine, or alternatively from the corresponding formamide (vallesine), contains one C-methyl grouping[5]; the isolation of propionic acid in the Kuhn–Roth determination[3d,6] indicates that this is actually a C-ethyl grouping. The molecule apparently contains no additional centers of unsaturation, so its composition taken together with I implies a pentacyclic structure.




The proton magnetic resonance spectra of aspidospermine (curves A and B) show peaks at 980 c.p.s.[7] for the aromatic hydrogen atoms, at 1100 c.p.s. for the O-methyl, 1168 c.p.s. for the acetyl C-methyl and 1227 c.p.s. for the ethyl C-methyl; the multiplets near 1200 c.p.s. are due to the various C-methylenes. The spectrum (curve C) of N-acetylaspidosine (II, R = Ac) does not contain the O-methyl peak while the curve (D) for deacetylaspidospermine does not contain the intense 1168 c.p.s. maximum. The presence of the strong resonance at 1164 c.p.s., midway between the peaks due to the O-methyl and the C-methyl suggested the possibility of an N-methyl.[1,8] It has been stated,[2,5] that the alkaloid contains no N-methyl group; although Djerassi, *et al.*, have recently reported low N-methyl values for aspidospermine (calcd. for one N-methyl, 4.22; found,[9] 1.83, 0.50),

(1) Part I of this series appears as a preliminary Communication, H. Conroy, P. R. Brook, M. K. Rout and N. Silverman, THIS JOURNAL, **79**, 1763 (1957).

(2) A. J. Ewins, *J. Chem. Soc.*, **105**, 2738 (1914).

(3) (a) H. T. Openshaw, G. F. Smith and J. R. Chalmers, XIIIth International Congress of Pure and Applied Chemistry, Stockholm and Uppsala, 1953, Abstracts p. 223; (b) H. T. Openshaw and G. F. Smith, *Experientia*, **4**, 428 (1948); (c) J. R. Chalmers, H. T. Openshaw and G. F. Smith, *J. Chem. Soc.*, 1115 (1957); (d) A. J. Everett, H. T. Openshaw and G. F. Smith, *ibid.*, 1120 (1957).

(4) (a) B. Witkop, THIS JOURNAL, **70**, 3712 (1948); (b) B. Witkop and J. B. Patrick, *ibid.*, **76**, 5603 (1954).

(5) E. Schlittler and M. Rottenberg, *Helv. Chim. Acta*, **31**, 446 (1948).

(6) W. I. Taylor, THIS JOURNAL, **79**, 3298 (1957); M. F. Bartlett, D. F. Dickel and W. I. Taylor, *ibid.*, **80**, 126 (1958).

(7) At 40.01 mc./sec. on an arbitrary scale wherein the toluene aromatic resonance peak is assigned a value of 1000 c.p.s. and the toluene methyl proton peak assigned 1197 c.p.s. Spectra, except for curve B, were examined in chloroform solution with a toluene capillary for external reference on a Varian Associates high resolution nuclear magnetic resonance spectrometer with superstabilizer.

(8) N-Methyl resonance would be expected to occur in this vicinity on the basis that chemical shifts are dependent upon the electronegativity of adjacent atoms. Empirical observations with compounds known to contain that system, with the exception of cases wherein the nitrogen is conjugated with electron-withdrawing groups as in amides, have generally supported the plausibility of the assignment. In carbon tetrachloride or chloroform solution these N-methyl peaks were observed: β-dimethylaminoethyl alcohol, 1169, and dimethylaniline, 1139 (A. A. Bothner-By, private communication); dimethylformamide, 1141, 1148; dimethylformamide neat, 1155, 1162; and dimethylcyanamide neat, 1153 (B. Bonne, M. A. Thesis, Brandeis University, 1957); thebaine, 1160; gelsemine, 1164 c.p.s.

(9) O. O. Orazi, R. A. Corral, J. S. E. Holker and C. Djerassi, *J. Org. Chem.*, **21**, 979 (1956).