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ALKALOID STUDIES—XXI*

PARTIAL STRUCTURE OF CASIMIROEDINE†

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Abstract—Degradation experiments are reported which demonstrate that the alkaloid casimiroedine contains the N-cinnamoyl-N-methyl-histamine moiety.

CASIMIROEDINE, the principal alkaloid of the seeds of *Casimiroa edulis* La Llave et Lej., has already been isolated in 1911,[1] but its correct empirical formula ($C_{21}H_{27}N_3O_6$) has been established only very recently.[2,3] Preliminary characterization experiments[3] involving acid or basic hydrolysis demonstrated that casimiroedine is the cinnamic acid amide of a base ($C_{12}H_{21}N_3O_5$)—named casimidine—and it has now been possible to gain further insight into the structure of this interesting alkaloid.

As has already been reported earlier,[3] casimiroedine and some of its derivatives consume 2 moles of periodic acid and this reaction has now been carried out on a preparative scale with dihydrocasimiroedine.§ Initial difficulties in preparing an analytically pure sample of the resulting amorphous amphoteric product ($C_{20}H_{25}N_3O_5$)—apparently due to incorporation of small amounts of iodine[4]—led to purification attempts involving treatment with zinc in acetic acid. Such a procedure afforded material which gave satisfactory analyses but occasionally such zinc treatment provided in poor yield a second substance corresponding to $C_{15}H_{19}N_3O$. The paucity of oxygen atoms in the latter product was greatly encouraging since many of the experimental difficulties—particularly with casimidine—are due to the large number of heteroatoms. Whereas barium hydroxide hydrolysis of dihydrocasimiroedine results[3] simply in cleavage of the amide linkage with formation of dihydrocinnamic acid and the oxygen-rich casimidine ($C_{12}H_{21}N_3O_5$), similar treatment of the periodic acid cleavage products $C_{20}H_{25}N_3O_5$ and $C_{15}H_{19}N_3O$ provided dihydrocinnamic acid and two *oxygen-free* bases. Separation of the bases was achieved by

* Paper XX, C. Djerassi, T. Nakano and J. M. Bobbitt *Tetrahedron* **2**, 58 (1958).

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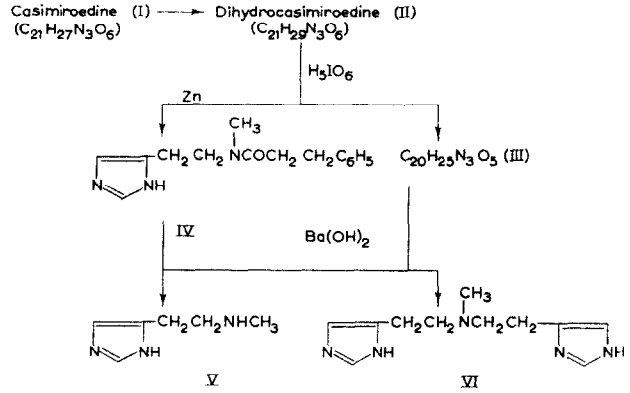
§ Dihydrocasimiroedine shows more favorable solubility characteristics and hence was selected as the starting material instead of the parent alkaloid. As demonstrated earlier[3] hydrogenation involves only saturation of the double bond of cinnamic acid without affecting the rest of the molecule.

[1] F. B. Power and T. Callan *J. Chem. Soc.* 1993 (1911).
[2] A. Aebi *Helv. Chim. Acta* **39**, 1495 (1956).
[3] C. Djerassi, J. Herran, H. N. Khastgir, B. Riniker and J. Romo *J. Org. Chem.* **21**, 1510 (1956).
[4] K. Hofmann *Imidazole and its Derivatives.* Interscience, New York (1953).

the genus *Casimiroa* belongs to the same plant family and more strikingly, that N, N-dimethylhistamine has recently been encountered[8] in *Casimiroa edulis*.



EXPERIMENTAL*

Periodic acid oxidation of dihydrocasimiroedine (II). A solution of 4·4 g of dihydrocasimiroedine (II)[3] in 80 cm³ of water containing 8·8 g of periodic acid was left at room temperature for 4 hr and the pH of the solution was then adjusted to 6·5† with aqueous sodium hydroxide. Continuous extraction with chloroform for 20 hr yielded 3·75 g of amber-colored oil which could not be crystallized. Chromatography in chloroform solution on Merck acid-washed alumina and washing with the same solvent furnished a trace of material while the bulk was eluted with chloroform–methanol (10 : 1) and represented a colorless foam resistent to crystallization. Homogeneity was demonstrated by the virtual identity of the infrared spectra of various fractions eluted with chloroform–methanol (10 : 1) and in particular by countercurrent distribution. A distribution coefficient of unity was obtained in a solvent system consisting of chloroform–hexane (28 : 12) in the lower phase and methanol–water (3 : 1) in the upper one. Each layer consisted of 25 cm³ and a 28-tube all-glass countercurrent distribution apparatus was employed. The peak fractions (16–19) exhibited the same infrared spectrum as the original chromatogram fractions but for further purification, they were rechromatographed and the amorphous product was dried for 20 hr at 60° under high vacuum.

Anal. Calcd. for $C_{20}H_{25}N_3O_5$: C, 62·00; H, 6·50; N, 10·85; O, 20·65. Found: C, 59·43; H, 6·73; N, 10·83; O, 19·18.

A 1·3 g sample of the above material in 120 cm³ of acetic acid was stirred for 2 hr at 30–40° with 8 g of zinc dust and the resulting product was chromatographed twice before drying for 48 hr at 60° and 0·01 mm; colorless amorphous material liquefying near 125°, no infrared absorption bands between 3·6 and 6·0 μ, $[\alpha]_D$ −3° (*c*, 0·72 in $CHCl_3$), −5° (*c*, 0·68 in 1% HCl), −15° (*c*, 0·7 in 1% NaOH).

* Melting points were determined on the Kofler block. The microanalyses were performed by Mr. Joseph F. Alicino (Metuchen, New Jersey) and Dr. A. Bernhardt (Mülheim, Germany). We are greatly indebted to Dr. Harold Boaz of Eli Lilly Co. (Indianapolis, Indiana) for the electrometric titrations which were carried out in 33% aqueous dimethyl formamide and to Dr. F. Kincl (Syntex, S.A., Mexico City) and Dr. F. A. Hochstein (Chas. Pfizer Co., Brooklyn, New York) for supplies of casimiroedine.

† The product was amphoteric and could not be extracted with chloroform from either a strongly acidic or strongly basic medium.

[8] F. A. Hochstein and J. Ling *New York Section Meeting-in-Miniature.* American Chemical Society (February 15, 1957).

Anal. Found: C, 61·29; H, 6·71; N, 10·70; 0, 20·88.

Subsequently, it was found that analytically pure material could be secured easily in the following manner. The oxidation mixture from 10 g of dihydrocasimiroedine, 11 g of periodic acid and 150 cm³ of water was adjusted to pH 1·8 after 4 hr at room temperature and was then extracted continuously overnight with chloroform. Evaporation of the extract furnished 0·43 g of reddish oil which was discarded. The pH of the aqueous solution was adjusted to 5·5 with 1 N sodium hydroxide and again extracted continuously with chloroform. Drying and evaporation of the organic layer provided 5·7 g of amorphous solid which was filtered in chloroform–methanol solution through alumina before being submitted to analysis; $[\alpha]$ −3° (*c*, 0·70 in $CHCl_3$), −5° (*c*, 0·85 in 1% HCl), −14° (*c*, 0·91 in 1% NaOH).

Anal. Found: C, 61·24; H, 7·06; N, 10·46; O, 20·68.

Isolation of N-*dihydrocinnamoyl*-N-*methylhistamine* (IV). When the zinc–acetic acid treatment of the periodic acid oxidation product (III) was conducted at 60°, chromatography gave in addition to pure III about 8–10 per cent of material which could be crystallized from methanol–ether to give colorless crystals of m.p. 134–135°, $\lambda_{max}^{CHCl_3}$ 6·10 μ, pK_a 6·5 (33% DMF), mol. wt. 240 ± 20 (by electrometric titration). The yield could not be improved on raising the reaction temperature or extending the time.

Anal. Calcd. for $C_{15}H_{19}N_3O$: C, 70·00; H, 7·44; N, 16·33; 0, 6·22. Found: C, 69·69; H, 7·43; N, 16·14; O, 6·69.

Isolation of N-*methylhistamine* (V) *and di-β-(4-ethylimidazolyl)-methylamine* (VI). A solution of 5·7 g of III in 80 cm³ of 10% aqueous barium hydroxide (octahydrate) was heated under reflux for 24 hr. After removal of some insoluble material, the clear, colorless solution was saturated with carbon dioxide, the precipitated barium carbonate was collected and the filtrate was acidified with hydrochloric acid. Extraction with ether furnished 2·06 g of dihydrocinnamic acid, m.p. 40–42°, while addition of a methanolic picric acid solution to the aqueous filtrate led to a yellow picrate. Trituration with hot methanol and filtration left 300 mg of insoluble picrate (m.p. 220°), which after four recrystallizations from acetone exhibited m.p. 226–228° (dec.) and which was identified as the tripicrate of di-β-(4-ethylimidazolyl)-methylamine (VI)* by mixture melting point determination and infrared comparison with a synthetic specimen.

Anal. Calcd. for $C_{29}H_{26}N_{14}O_{21}$: C, 38·42; H, 2·89; N, 21·63; 0, 37·06. Found: C, 38·10; H, 2·90; N, 21·86; 0, 37·68.

Cooling of the hot methanol washes of the crude picrate led to 1·5 g of crystals, m.p. 180–187°, which were purified most effectively by chromatography, since Merck acid-washed alumina did not decompose the picrate. A 1·0 g sample of the picrate was chromatographed on 30 g of alumina and eluted with acetone and with methanol–water (9 : 1) to give 0·92 g of material with m.p. 185–188°. The analytical sample was recrystallized from methanol whereupon it exhibited m.p. 187–188°, undepressed upon admixture with a synthetic specimen of the dipicrate of N-methylhistamine (V); the infrared spectra in Nujol mull were also identical. The same dipicrate (135 mg, m.p. 188–189°) was isolated when 320 mg of IV was treated with barium hydroxide in the above described fashion.

* The hydrochloride, m.p. 231–234°, was hygroscopic and was not submitted for analysis. However, it did not give a melting point depression when mixed with a synthetic sample of the hydrochloride.

Anal. Calcd. for $C_{18}H_{17}N_9O_{14}$: C, 37·06; H, 2·94; N, 21·61; O, 38·39; N–CH_3, 2·56; mol. wt., 583·4. Found: C, 37·42; H, 3·06; N, 21·86; O, 37·60; N–CH_3, 2·01; neut. equiv. (perchloric acid titration), 283.

Alternatively, 1·45 g of III was heated under reflux for 20 hr with 1 : 1 aqueous hydrochloric acid and ether extraction removed 0·76 g of dihydrocinnamic acid. Evaporation of the aqueous solution to dryness gave a gummy hydrochloride which was treated in aqueous solution with picric acid. A brown, oily picrate was formed which yielded after repeated treatment with hot methanol 0·3 g of yellow picrate, m.p. 180–188°. The free base was regenerated by passing an acetone solution (containing a few drops of water which increased the solubility of the salt) of the picrate through a column of basic IRA–400 Amberlite resin and eluting rapidly with methanol to avoid base-catalyzed condensation of acetone. Evaporation of the eluates to dryness led to N-methylhistamine (V) as a colorless, odorless, viscous oil which was distilled twice at 0·005 mm and a bath temperature of 170°. The oil showed no selective ultraviolet absorption above 220 mμ but exhibited a blue fluorescence when exposed to an ultraviolet lamp very similar to 4-methylimidazole (prepared from the picrate which was kindly provided by Dr. C. F. Huebner of Ciba Pharmaceutical Products, Inc., Summit, New Jersey). Considerable similarities were also observed in the infrared spectra of 4-methylimidazole and N-methylhistamine.

Anal. Calcd. for $C_6H_{11}N_3$: C, 57·57; H, 8·86. Found: C, 57·58; H, 8·28; C–CH_3, 0·52.*

Treatment of 58 mg of the base with 140 mg of picric acid in methanol solution yielded 108 mg of N-methylhistamine dipicrate, m.p. 187–189°.

Anal. Calcd. for $C_{18}H_{17}N_9O_{14}$: C, 37·06; H, 2·94; N, 21·61. Found: C, 37·37; H, 2·86; N, 21·66.

When a solution of 115 mg of styphnic acid in 3 cm^3 of methanol was added to 45 mg of N-methylhistamine (V) dissolved in 2 cm^3 of the same solvent, an immediate precipitate of the monostyphnate of N-methylhistamine separated. The substance was very insoluble in methanol and had to be recrystallized from acetone–methanol, whereupon it exhibited m.p. 235–245° (very dependent upon rate of heating).

Anal. Calcd. for $C_{12}H_{14}N_6O_8$: C, 38·92; H, 3·81; N, 22·70. Found: C, 38·87; H, 4·17; N, 22·99.

Monostyphnate formation (in contrast to the above described dipicrate) was clearly due to the insolubility of the salt in methanol and a distyphnate could indeed be prepared when the base and styphnic acid were mixed in water solution and then recrystallized from ethanol; yellow needles, m.p. 203–204°.

Anal. Calcd. for $C_{18}H_{17}N_9O_{16}$: C, 35·13; H, 2·79; N, 20·50. Found: C, 34·97; H, 2·74; N, 20·87.

Synthesis of N-*methylhistamine* (V) *and di-*β*-(4-ethylimidazolyl)-methylamine* (VI). A solution of 2·0 g of 4-(β-chloroethyl)-imidazole hydrochloride, prepared from butyne-1, 4-diol according to the procedure of Huebner,[6] in 20 cm^3 of 10% methanolic methylamine was heated in a sealed tube for 15 hr at 95–100°. The contents were cooled, evaporated to dryness *in vacuo*, taken up in a small amount of water and treated with a methanolic solution of 0·62 g of picric acid. The picrate was crystallized from a large amount of methanol and three crops were collected: (a) 252 mg, m.p.

* In order to observe the ease of C-methyl determination in the imidazole series, a sample of 4-methylimidazole was submitted to Kuhn-Roth analysis and yielded 15·5 per cent C–CH_3 (calcd., 18·3 per cent).

200–210°; (b) 86 mg, m.p. 181–215°; (c) 172 mg, m.p. 177–179°. Several recrystallizations of the first crop from acetone raised the m.p. of di-β-(4-ethylimidazolyl)-methylamine tripicrate (VI) to 225–227°.

Anal. Calcd. for $C_{29}H_{26}N_{14}O_{21}$: C, 38·42; H, 2·89. Found: C, 38·63; H, 3·17.

The hydrochloride, m.p. 233–235°, was hygroscopic and was not analyzed.

Recrystallization of the third picrate crop from methanol yielded N-methylhistamine dipicrate, m.p. 187–189° (in reference 5, m.p. 188°).

Anal. Calcd. for $C_{18}H_{17}N_9O_{14}$: C, 37·06; H, 2·94; O, 38·39. Found: C, 37·40; H, 3·20; 0, 38·57.