Notes

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Alkaloid Studies. XV.[1] Casimiroedine

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Received July 17, 1956

The seeds from the fruit ("zapote blanco") of the Mexican tree *Casimiroa edulis* La Llave et Lex. appear to have been used in indigenous medicine for a long time[3] and our own interest in this plant was stimulated by the reported[4] isolation from it of a new crystalline alkaloid, casimiroedine. Quite recently,[5] there has been described a very thorough chemical investigation of the seeds from which a remarkable variety of products were obtained.

Power and Callan[4] did not carry out any structural investigations on casimiroedine other than to determine its analytical composition from which they arrived at the empirical formula $C_{17}H_{24}N_2O_5$. We encountered no difficulty in isolating casimiroedine by substantially the same scheme outlined by the British authors but in agreement with Aebi[6] we assign to the alkaloid the formula $C_{21}H_{27}N_3O_6$.

The relevant ultraviolet and infrared spectroscopic data have already been given by Aebi[6] and it was pointed out by him that in addition to NH and/or OH absorption, there was observed a strong band at 6.08 μ ascribable to an amide grouping. The presence of one C-methyl group and four active hydrogen atoms but the absence of methoxyl or acetyl functions was noted and the alkaloid was characterized further as the tetra-acetate and as the tetrahydro derivative. Prompted by Aebi's paper[6] we should like to describe briefly some preliminary chemical data which suggest the more profitable routes to be undertaken for the complete structure elucidation of this interesting alkaloid.[7]

Catalytic hydrogenation of casimiroedine with palladium-charcoal led to dihydrocasimiroedine which because of its increased solubility was more suitable for most degradations. Perchloric acid titration indicated only one titratable nitrogen atom and casimiroedine formed only a mono-picrate. Strong acid or alkaline hydrolysis of casimiroedine yielded cinnamic acid (accounting for the observed ultraviolet absorption spectrum of the alkaloid) while dihydrocasimiroedine[8] furnished dihydrocinnamic acid. The other hydrolysis product in each case was a water-soluble, crystalline base ("casimidine") corresponding to $C_{12}H_{21}N_3O_5$ (two titratable nitrogen atoms) which could be characterized further as the dihydrochloride and as a dipicrate. It is clear, therefore, that the alkaloid casimiroedine is the cinnamic acid amide[9] of casimidine. Casimiroedine, dihydrocasimiroedine as well as casimidine are very rapidly oxidized by means of periodic acid with consumption of two equivalents of reagent and without the liberation of any volatile aldehyde. Further degradation experiments with the amphoteric periodic acid oxidation product are now in progress.

Aebi[6] has reported the presence of one C-methyl group in the alkaloid. Since Kuhn-Roth oxidations of casimiroedine carried out in three different analytical laboratories differed greatly, we have carried out such an oxidation on a preparative scale and were unable to isolate any acetic acid. The entire volatile, titratable acid proved to be benzoic acid (produced by oxidation of cinnamic acid) and it appears that varying quantities of benzoic acid carried over during the micro Kuhn-Roth determination are responsible for the observed C-methyl values. In accordance with this observation, casimidine did not show any C-methyl group in the Kuhn-Roth determination. We have confirmed the

(1) Paper XIV, C. Djerassi, S. K. Figdor, J. M. Bobbitt and F. X. Markley, *J. Am. Chem. Soc.*, **78**, 3861 (1956).

(2a) Squibb Postdoctorate Research Fellow, 1952–1953; (b) Pfizer Postdoctorate Research Fellow, 1955–1956.

(3) *Inter al.*, M. Martinez, *Plantas Medicinales de Mexico*, Ediciones Botas, Mexico, D. F., 1944, pp. 326–330; L. Vincent, *Bull. Gen. Therap.*, **158**, 193 (1909); M. J. Chevalier, *Bull. Gen. Therap.*, **158**, 96 (1909). It has been suggested by V. A. Reko (*Magische Gifte*, Ferdinand Enke Verlag, Stuttgart, 1938, 2nd. Ed., pp. 148–153) that some of the reported hypnotic effects are actually caused by "zapote borracho" (*Lucuma salicifolia* H.B.K.) with which "zapote blanco" may have been confused.

(4) F. B. Power and T. Callan, *J. Chem. Soc.*, 1993 (1911).

(5) F. A. Kincl, J. Romo, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 4163 (1956).

(6) A. Aebi, *Helv. Chim. Acta*, **39**, 1495 (1956). We are greatly indebted to Dr. Aebi for his courtesy in sending us a copy of his paper prior to publication.

(7) Both Power and Callan (ref. 4) and Aebi (ref. 6) report the pharmacological inactivity of casimiroedine.

(8) It is interesting to note that while casimiroedine exhibits infrared bands at 6.09 and 6.32 μ, the dihydro derivative shows only a single band at 6.20 μ.

(9) Naturally occurring amides of cinnamic acid have already been observed earlier (*cf.* F. B. LaForge and W. F. Barthel, *J. Org. Chem.*, **9**, 250 (1944); I. Ribas, R. Guitian and P. Taladrid, *Anales real soc. espan. fis. y quim.*, **47B**, 715 (1951)).

reported[4,6] absence of methoxyl but have observed that one N-methyl group is present.

EXPERIMENTAL[10]

Casimiroedine. The isolation of the alkaloid was carried out by a modification of the Power and Callan scheme[4] but in view of Aebi's improved directions[6] this will not be reported. The analytical sample was crystallized from ethanol whereupon it was obtained as colorless crystals, m.p. 223–224°, $[\alpha]_D$ −27° (1% HCl), λ_{max}^{Nujol} 2.97, 6.09, 6.32 and sharp, strong band at 9.0 μ, λ_{max}^{EtOH} 219 and 280 mμ, log ϵ 4.26 and 4.30.

Anal. Calc'd for $C_{21}H_{27}N_3O_6$: C, 60.42; H, 6.52; N, 10.07; Mol. wght., 417. Found: C, 60.38; H, 6.83; N, 9.81 (no amino nitrogen present); Methoxyl, 0.0; Neut. equiv., 389 (perchloric acid titration), *ca.* 450 by electrometric titration (in 25% aqueous dimethylformamide solution), *p*k'a, 5.0.

The absence of a C-methyl group was demonstrated by heating under reflux a solution of 1.5 g. of casimiroedine in 100 cc. of water and 20 cc. of conc'd sulfuric acid and adding 20 g. of chromium trioxide in four portions over a period of 1.5 hours. After two hours, the contents were subjected to steam-distillation and the first three 150-cc. distillates were titrated (phenolphthalein) with 0.2 *N* sodium hydroxide, requiring respectively 17, 5, and 1 cc. of base. The first portion was evaporated to dryness and the non-identity of the residue with sodium acetate was established by the fact that it did not melt below 350°. Solution of the residue in water, acidification with sulfuric acid and ether extraction yielded 160 mg. of pure benzoic acid.

Casimiroedine picrate crystallized with difficulty from methanol as yellow needles, m.p. 110–112°.

Anal. Calc'd for $C_{27}H_{30}N_6O_{13}$: C, 50.87; H, 4.71; N, 13.19. Found: C, 50.43; H, 4.79; N, 12.61.

Casimiroedine tetrabenzoate. A one-gram sample of casimiroedine was dissolved in 10 cc. of 5% sulfuric acid, made alkaline with 50 cc. of 30% sodium hydroxide solution and then shaken vigorously for 30 minutes with 4 cc. of benzoyl chloride. After cooling in ice, the precipitate (1.21 g.) was collected and washed well with water. The analytical sample was obtained by repeated precipitation from dilute methanol but no sharp m.p. could be realized (m.p. 97–105°) $[\alpha]_D$ +29° (ethanol), $\lambda_{max}^{CHCl_3}$ 5.79, 6.08, 6.23 and broad band at 7.95 μ.

Anal. Calc'd for $C_{49}H_{43}N_3O_{10}$: C, 70.69; H, 5.20; N, 5.04. Found: C, 70.35; H, 5.61; N, 4.83.

Dihydrocasimiroedine. Casimiroedine (10.0 g.) was dissolved by warming in 400 cc. of methanol-water (5:1) and then was hydrogenated at room temperature and atmospheric pressure with 500 mg. of 10% palladized charcoal catalyst. Filtration of the catalyst, evaporation to dryness and recrystallization from benzene–methanol produced 9.8 g. of colorless crystals of dihydrocasimiroedine, m.p. 176–177°, $[\alpha]_D$ +12° (ethanol), λ_{max}^{Nujol} 2.93, 6.20 and 9.0 μ.

Anal. Calc'd for $C_{21}H_{29}N_3O_6$: C, 60.13; H, 6.97; N, 10.02; N—CH_3, 3.6; Mol. wght., 419. Found: C, 59.94; H, 7.03; N, 9.98; OCH_3, 0.0; N—CH_3, 3.92; Neut. equiv., 410 (perchloric acid titration); *p*K'a 5.0 (25% aqueous dimethylformamide).

Acid hydrolysis of casimiroedine. A solution of 1.0 g. of the alkaloid in 20 cc. of 6 *N* hydrochloric acid was heated under reflux for 8–11 hours and cooled whereupon a crystalline precipitate separated. This was extracted with ether and after processing in the usual manner there was obtained 0.285 g. (78%) of *cinnamic acid* with m.p. 133–134°, undepressed upon admixture with authentic material. The aqueous acid solution was evaporated to dryness in a desiccator and the residue was crystallized several times from absolute methanol to afford small, colorless needles of *casimidine dihydrochloride*, m.p. 198–200°.

Anal. Calc'd for $C_{12}H_{23}Cl_2N_3O_5$: C, 40.01; H, 6.44; N, 11.67; Cl, 19.69; Mol. wght., 360. Found: C, 39.66; H, 6.71; N, 11.54; Cl, 19.36; Neut. equiv., 172.

Crystalline *casimidine*[11] was best prepared by passing a methanol solution of the dihydrochloride through a column of Amberlite IRA-400 and concentrating the eluates to a small volume.[12] The analytical sample crystallized from *absolute* methanol (casimidine is quite soluble in water) as colorless prisms, m.p. 207–209°, $[\alpha]_D$ +11° (80% ethanol), no carbonyl bands in the infrared.

Anal. Calc'd for $C_{12}H_{21}N_3O_5$: C, 50.16; H, 7.37; N, 14.63; N—CH_3, 5.23; Mol. wght., 287. Found: C, 50.56; H, 7.45; N, 14.52; N—CH_3, 5.03; C—CH_3, 0.0; Neut. equiv. (perchloric acid titration), 148.

Casimidine dipicrate was prepared by dissolving the base (or its hydrochloride) and picric acid in alcohol and adding water to incipient turbidity. The analytical sample crystallized from dilute ethanol as bright yellow crystals, m.p. 130°, resolidifying at 50° and finally melting at 174–176°.

Anal. Calc'd for $C_{24}H_{27}N_9O_{19}$: C, 38.66; H, 3.65; N, 16.91; Mol. wght., 745. Found: C, 38.86; H, 3.82; N, 16.58; Neut. equiv. (perchloric acid titration), 373.

Acid hydrolysis of dihydrocasimiroedine. The reaction proceeded in essentially the same yield as described above for casimiroedine and yielded casimidine dihydrochloride and dihydrocinnamic acid, m.p. 41–42°. Identity of the latter was established by mixture melting point determination of the acid as well as of the amide (m.p. 103–105°) with authentic specimens.

Basic hydrolysis of casimiroedine. Casimiroedine (2.0 g.) was heated under reflux with 20 cc. of a saturated solution of barium hydroxide for 20 hours and then neutralized with Dry Ice. The barium carbonate was filtered and the filtrate was extracted with ether to furnish 0.62 g. (87%) of cinnamic acid. The aqueous layer was evaporated to dryness *in vacuo* yielding 1.05 g. of crude casimidine which melted at 205–208° after one recrystallization from absolute methanol.

Periodic acid oxidations. The preparative experiments will be described at a later date together with the chemistry of the oxidation products. The periodic acid oxidations were carried out in aqueous solution, dihydrocasimiroedine dissolving immediately in the aqueous medium. Within 10 minutes 80% of the calculated amount (for two equivalents) was consumed and 96.5% were used up after 35 minutes. When casimidine was oxidized under the same conditions, 77.5% of reagent was used in 5 minutes and 97% of the theoretical amount (based on 2 equivalents) in 30 minutes.

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(10) Melting points are uncorrected. Infrared spectra were determined on a Baird double beam infrared spectrophotometer. Analyses were carried out by Mr. Joseph F. Alicino (Squibb Institute for Medical Research), Spang Microanalytical Laboratory (Plymouth, Michigan) and Geller Laboratories (Hackensack, New Jersey).

(11) The crystalline base was obtained for the first time in this laboratory by R. Mirza.

(12) The hydrochloride need not be purified prior to crystallization of the casimidine. Passage of the crude hydrochloride (obtained directly from the acid hydrolysis of 7.0 of casimiroedine) through Amberlite yielded 3.5 g. of casimidine satisfactory for further work.