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ISOLATION AND STRUCTURE OF CROTALARINE, A NEW ALKALOID FROM CROTALARIA BURHIA BUCH.-HAM.

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Abstract. Crotalarine, $C_{18}H_{27}NO_6$, m.p. 167–68°, $[\alpha]_D^{26}$—79.8° (ethanol), a new alkaloid isolated from the aerial parts of the plant *Crotalaria burhia* was hydrogenated in the presence of platinum oxide catalyst to tetrahydrocrotalarine, the properties of which indicated it to be a salt. Crotalaric acid was obtained from the salt. By alkaline degradation crotalarine gave *s*-butyl methyl ketone, DL-lactic acid and retronecine. Crotalarine absorbed one mole equivalent of periodic acid in 25–30 min and formed a cyclic sulphite ester with thionyl chloride. The properties and IR spectra of crotalarine, tetrahydrocrotalarine and crotalaric acid resembled closely those of trichodesmine, tetrahydrotrichodesmine and trichodesmic acid. A structure formula is proposed which satisfies all the properties of this alkaloid.

A pyrrolizidine alkaloid, $C_{18}H_{27}NO_6$, m.p. 167–68°, $[\alpha]_D^{26}$–79.8° (ethanol), tentatively named crotalarine, has been isolated from the aerial parts of the plant *Crotalaria burhia* Buch.-Ham. The IR spectrum of the alkaloid (in KBr) was very similar to that of trichodesmine,[1] with which it is isomeric, and showed hydroxyl absorption at 3425 cm^{-1} and carbonyl absorptions at 1724 and 1709 cm^{-1} (shoulder) seemingly due to ester linkages.

On periodic acid oxidation, crotalarine consumed one mole equivalent of the reagent indicating the presence of a vicinal glycol system in the alkaloid (*cf.* trichodesmine and monocrotaline[1]).

On alkaline hydrolysis, crotalarine yielded retronecine, $C_8H_{13}NO_2$, *s*-butylmethylketone and lactic acid in addition to a comparatively much smaller amount of a white crystalline solid, m.p. 234–35°. This compound was found to have the molecular formula, $C_{10}H_{16}O_5$, and showed a striking resemblance to trichodesmic acid and monocrotalic acid[1] in the IR absorption spectrum which suggested a close similarity in structure. Absorptions at1748, 1712 and 3472 cm^{-1} in the IR spectrum of this compound (in KBr) were indicative of a 5-membered lactone ring, a normal carboxyl and an alcoholic hydroxyl group respectively. Results of direct titrations with alkali confirmed this compound to be a monobasic acid containing one lactone ring. We suggest the name crotalaric acid for this compound.

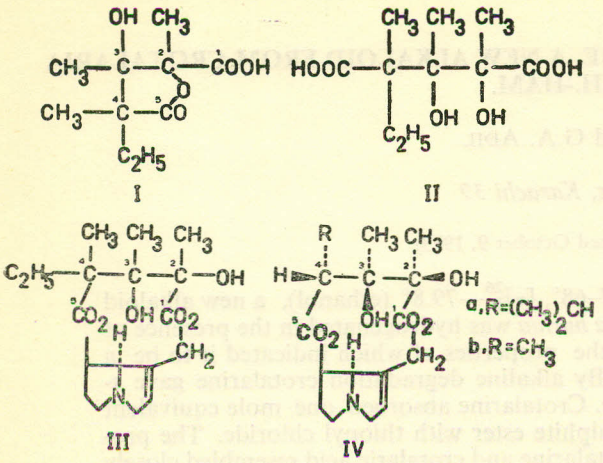
Crotalarine, on hydrogenation over PtO_2 catalyst, gave a crystalline tetrahydro derivative, $C_{18}H_{31}NO_6$, m.p. 237–41°. Its IR spectrum (KBr) showed the complete absence of ester carbonyls, but the presence of bands at 1600 ($C(=O)-O^-$), 2564 ($-N^+-H$) and 1755 cm^{-1} (γ-lactone). The anion and cation portions of this salt are thus separate—a fact which was verified by using a sulphonic ion exchanger. The cation portion of the salt was removed and the acid left was identified as crotalaric acid.

The formation of crotalaric acid (a C_{10} compound) on hydrolysis as well as hydrogenolysis of crotalarine, and at the same time absence of a lactonic absorption in the IR spectrum, made it clear that crotalarine was a cyclic diester of retronecine, the two ester linkages being of different nature—one normal (1709 cm^{-1}) and the other allylic (1724 cm^{-1}). The other hydrolysis products of crotalarine, namely *s*-butyl methyl ketone and lactic acid, suggested that necic acid component of crotalarine was of a substituted glutaric acid type as occurring in trichodesmine and monocrotaline which, notably, under similar conditions of hydrolysis, yield isobutyl methyl ketone and ethyl methyl ketone respectively, in addition to lactic acid in the former case.[1–3] A mechanism for this degradation has been suggested by Adams and Gianturco.[1] Furthermore, the formation of the lactone ring in crotalaric acid upon hydrogenolysis, necessitates the presence of a hydroxyl group on the carbon atom alpha to the carboxyl group produced by the hydrogenolysis of the allylic ester linkage.[4] All these facts led us to formulate crotalaric acid as 2,3-dihydroxyl-2,3,4-trimethyl-4-ethyl glutaric acid, 2(γ)-lactone (I) which was derived from the necic acid (II). It is well-conceived that, on alkaline hydrolysis of crotalarine, compound (II) is first formed which subsequently undergoes lactonisation to yield crotalaric acid. *s*-Butyl methyl ketone and lactic acid result from the degradation of compound (II).

Knowing the structure of the necic acid component as 2,3-dihydroxyl-2,3,4-trimethyl-4-ethyl glutaric acid (II) and the relative positions of its hydroxyls with respect to the carboxyl involved in the formation of the allylic ester with retronecine, the structure of crotalarine is clearly established as shown in III.

The NMR spectrum of crotalarine has been found to be in complete agreement with the following structure.

The proof for the *cis*-configuration of the two hydroxyl groups present at C-2 and C-3 in trichodesmine (IVa) and monocrotaline (IVb), which are structurally analogous to crotalarine, is based on the formation of cyclic sulphite esters by these compounds on reaction with thionyl chloride.[5,6] Crotalarine and thionyl chloride gave a colourless crystalline product, m.p. 204°, with the correct analysis, $C_{18}H_{26}ClNO_7S$, for the hydrochloride of cyclic sulphite ester of the alkaloid.



The two hydroxyl groups in crotalarine should, therefore, possess the *cis*-configuration.

Experimental

The solvent used in paper chromatography (ascending technique) was the upper phase resulting from shaking equal volumes of n-butanol with 5% acetic acid. Dragendorff's reagent (10%) was used to develop the chromatograms. IR spectra were recorded with a Perkin–Elmer 237 grating spectrometer and NMR spectra (60 mc/sec) with TMS as an internal reference.

Extraction and Isolation of Crotalarine. The fresh plant (6 kg) was soaked in ethanol and kept for fifteen days. The extract was concentrated under reduced pressure and to the residue added 500 ml petroleum ether (66–68°) and 200 ml distilled water. Separated the aqueous layer, washed with 50 ml chloroform and reduced the *N*-oxide with 0.5N H_2SO_4 and zinc dust. Filtered the reduced extract, basified to pH 10 with NH_4OH (concd) and extracted six times with chloroform using 100 ml each time. Dried the chloroform over Na_2SO_4 (anhydrous), filtered and removed the solvent completely under reduced pressure. The crude base (yield 0.066%) showed two spots *Rf* 0.58 and 0.68 on the paper chromatogram (Whatman No. 1), crotalarine having *Rf* 0.58 was in major quantity and purified by fractional crystallisation using dry benzene, m.p. 167–8°. (Found: C, 61.54; H, 7.8; N, 3.94. Calc. for $C_{18}H_{27}NO_6$: C, 61.17; H, 7.7; N, 3.96%).

Derivatives. Three derivatives were prepared.

Picrate: Crystallised from abs. alcohol (dec. 240°) (Found: C, 49.47; H, 5.18; N, 9.48. Calc. for $C_{18}H_{27}NO_6.C_6H_3N_3O_7$: C, 49.48; H, 5.15; N, 9.62%).

Methiodide: Crystallised from methanol ether (m.p. 201–3°). (Found: C, 46.55; H, 6.35, N, 2.71; I, 25.95. Calc. for $C_{18}H_{27}NO_6.MeI$: C, 46.05; H, 6.06; N, 2.82; I, 25.66%).

Hydrochloride: Crystallised from abs. alcohol–ether, (m.p. 208–9°). (Found: C, 55.37; H, 7.43; N, 3.51; Cl, 9.34. Calc. for $C_{18}H_{27}NO_6.HCl$: C, 55.45; H, 7.19; N, 3.59; Cl, 9.11%).

Tetrahydrocrotalarine. A solution of 0.061 g crotalarine in 5 ml ethanol and 1 ml glacial acetic acid was hydrogenated at room temperature and atmospheric pressure with 0.006 g platinum oxide catalyst. Filtered to remove the catalyst, washed twice with abs. alcohol and eliminated the solvent under reduced pressure. A brownish residue was obtained, dissolved in dry acetone, filtered and crystallized using ethanol–ether (m.p. 237–41°). (Found: C, 61.11; H, 8.58. Calc. for $C_{18}H_{31}NO_6$: C, 60.48; H, 8.74%).

Crotalaric Acid. A solution of tetrahydrocrotalarine (10 mg) in 8 ml distilled water was prepared. Amberlite IR 120 (H) (2 g) was washed with water to remove any acidic portion. The aqueous solution was added to the resin, mixed thoroughly and decanted. Washed the resin with 5 ml water and combined the two portions. Eliminated the solvent under reduced pressure and crystallised using acetone–petroleum ether (b.p. 66–68°), m.p. 233–34° undepressed on admixture with the crotalaric acid obtained from alkaline hydrolysis.

Hydrolysis of Crotalarine. A solution of crotalarine (0.101 g) in 10 ml 10% NaOH (aq) was heated under reflux for 2 hr. Acidified to congo red with 5% HCl, extracted continuously with ether for 24 hr and dried (Na_2SO_4). Eliminated the solvent and the residue obtained showed a small amount of crystals (m.p. 234°C). Retronecine was isolated as its hydrochloride, m.p. 161–62°, undepressed on admixture with the authentic sample.

Hydrolysis of Crotalarine Using 1% NaOH. A solution of 0.31 g crotalarine in 18 ml 1% NaOH (aq) was heated under reflux using a very efficient condenser for 2 hr. The solvent was distilled off under reduced pressure and fractions were collected in 5 ml portions. 2,4-Dinitrophenylhydrazine reagent was added to the various fractions. The yellow ppt formed was filtered and crystallised from ethanol–water, m.p. 71–72° undepressed on admixture with an authentic sample of 2,4-dinitrophenylhydrazone of *s*-butyl methyl ketone. The residue was dissolved in 20 ml distilled water, acidified to congo red using 5% HCl. Extracted continuously with ether for 24 hr, dried (Na_2SO_4) and removed the solvent. A small amount of a syrupy substance was obtained which was identified as DL-lactic acid by IR spectra and paper chromatograms of known and unknown DL-lactice acid. Retronecine hydrochloride was isolated from the residue of the aqueous extract.

Periodic Acid Oxidation. A solution of 5×10^{-3} mole of crotalarine in 10 ml (0.02M) aqueous sodium periodate was prepared. The course of reaction was followed by withdrawing 1 ml aliquots of the solution and titrating the excess reagent by the arsenite method. Crotalarine absorbed one mole reagent in 25–30 min.

Reaction with Thionyl Chloride. Crotalarine (41 mg) was kept in an ice-bath for 15 min and thionyl chloride (1 ml) was added and mixed thoroughly. Kept at room temperature for 1 hr, removed the solvent under reduced pressure, washed with dry benzene and ether. Crystallised from ethanol–ether (m.p. 200–201°). (Found: C, 49.86; H, 5.99. Calc. for $C_{18}H_{27}NO_7S.HCl$: C, 49.59; H, 5.97%).

N M R Spectrum. The shift values recorded for different protons are: τ 3.76 (2 *H*), 4.65 and 5.05 (9 *H*),

8.41 and 8.45 (CH_3—C(OH)), 8.78 (CH_3—C) and 9.22 triplet (CH_3CH_2; *J* 7.8).

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