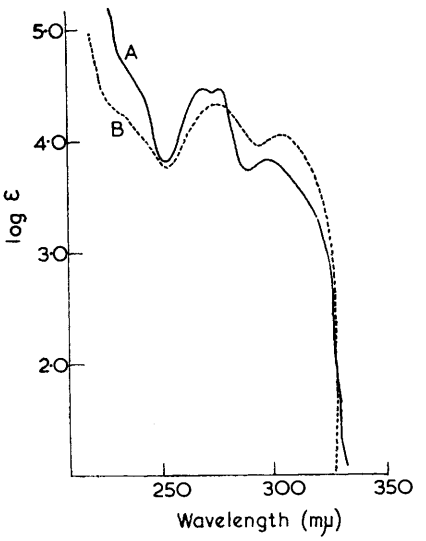
335. *Alkaloids from* Croton species. *Part II.*[1] *Structural Determination of Base A, Linearisine, and Crotonosine from* C. linearis *Jacq.*

By L. J. Haynes and K. L. Stuart.

Chemical and spectroscopic evidence is presented to support morphine-type skeletons, with *meta*-dihydric phenolic substitution, for crotonosine, base A, and linearisine.

Crotonosine, $C_{17}H_{17}NO_3$, was shown in Part I [1] to contain one methoxyl and one phenolic hydroxyl group, a secondary nitrogen atom, and two double bonds and a carbonyl group in a cross-conjugated dienone. The structure thus consists of four rings, one of which is aromatic.

Treatment of crotonosine with 3N-hydrochloric acid in methanol at 80° for 10 hr. yielded an amorphous rearrangement product which gave crystalline derivatives having ultraviolet absorption spectra characteristic of aporphine (see Figure). Analysis of these



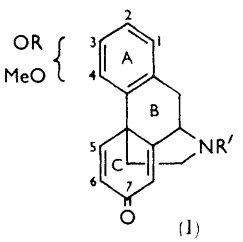
Absorption spectra of (A) *NO*-diacetyl-*O*-methyl-apocrotonosine and (B) morphothebaine.

derivatives, *NOO*-tri- and *NO*-di-methylapocrotonosine methiodide, and *NO*-diacetyl-*O*-methyl- and *N*-acetyl-*OO*-dimethyl-apocrotonosine, was consistent with the spectral evidence, as also was absence of hydrogenation in presence of reduced Adams catalyst. This rearrangement suggested that crotonosine has a morphine-type structure.[2] Supporting evidence was obtained by acetolysis [3] of crotonosine. The nitrogen-free product showed an ultraviolet spectrum characteristic of phenanthrene derivatives. This degradation is characteristic of the morphine–sinomenine group. It is then possible to accommodate the functional groups only in a structure of type (I; R = R′ = H).

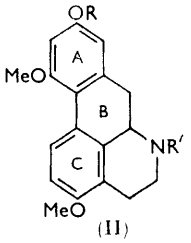
Evidence for the location of the phenolic hydroxyl and methoxyl groups first came from the nuclear magnetic resonance spectrum of *N*-acetyl-*OO*-dimethylapocrotonosine (II; R = Me, R′ = Ac). In the spectrum we assign peaks corresponding to τ 6·12, 6·18 and 6·32 to methoxyl groups at positions, 7, 2, and 4, respectively. The 7-methoxyl arises from the dienone–phenol rearrangement in acidic methanol. Assignment of a 4-methoxyl group follows from the correlation of the spectrum of aporphine alkaloids

[1] Part I, preceding paper.
[2] Mattiessen and Wright, *Proc. Roy. Soc.*, 1869, **17**, 455; *Ann. Suppl.*, 1870, **7**, 170; Howard, *Ber.*, 1884, **17**, 527; Small, Faris, and Mallonee, *J. Org. Chem.*, 1940, **5**, 334.
[3] Schryver and Lees, *J.*, 1900, **77**, 1038.

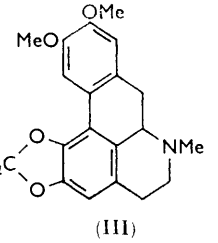
recently put forward by Bick *et al.*[4] and is supported by the observation that demethylapocrotonosine, m. p. 200—202°, prepared by heating crotonosine in glacial acetic acid with hydriodic acid, gave a positive Gibbs test with 2,6-dichloroquinone chloroimide. This shows that in crotonosine there is an unsubstituted position *para* to the methoxyl group. The assignment of the peak at τ 6·18 to a 2-methoxyl group follows mainly from a study of the resonance pattern produced by the hydrogen atoms on the aromatic rings. The spectrum in this area shows three main features. Single resonance bands at τ 3·35 and 2·91 represent *meta*-located protons in ring A. The doublets τ 1·96, 1·91 (J 3·9 c./sec.) and 3·19, 3·13 (J 3·9 c./sec.) represent an altered AB splitting pattern for the *ortho*-located 5- and 6-proton, respectively, in keeping with Shoolery's observation [5] that factors such as the electronegativity of adjacent groups and other types of deshielding affect coupling. The deshielding in this case has been discussed by Goodwin, Shoolery, and Johnson.[6] These authors demonstrated that a 4-proton in dicentrine (III) resonates at a much lower frequency than the remaining aromatic protons. This has been attributed to the fact that the dihedral angle between the two benzene rings is small and so results in deshielding. This is true of the 5-proton in *N*-acetyl-*OO*-dimethylapocrotonosine, and further deshielding is due to anisotropic effects of the 4-methoxyl groups. This leaves only position



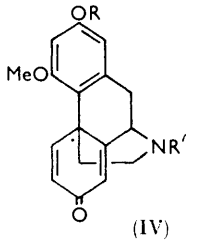
(I)



(II)



(III)



(IV)

2 for the third methoxyl group. This is in agreement with a τ value of 6·18. This 2-methoxyl group was derived from the original phenolic hydroxyl present in crotonosine, and this location is supported by a negative Gibbs test for crotonosine. The spectrum also showed *N*-acetyl resonance (τ 7·81) and ring-methylene (τ 7·26, 7·16); the entire spectrum is consistent with structure (II; R = Me, R′ = Ac).

Supporting evidence for a 4-methoxyl group in crotonosine was obtained by comparing the nuclear magnetic resonance spectra of *NO*-diacetylcrotonosine, *NO*-diacetyltetrahydrocrotonosine, and linearisine. The effect of hydrogenating the two double bonds in ring C was demonstrated by a shift in the methoxyl resonance from τ 6·47 in *NO*-diacetylcrotonosine to τ 6·12 in *NO*-diacetyltetrahydrocrotonosine. With one double bond in ring C [linearisine (V)], the methoxyl resonance was at 6·32 τ. This effect can be explained by the influence of π-electrons on the 4-methoxyl group and depends on the degree of shielding as one moves from a cross-conjugated dienone system to a $\alpha\beta$-unsaturated ketone. A 1-methoxyl group would not be so influenced.

Evidence favouring the assignment made on the basis of the nuclear magnetic resonance spectra was given by additional tests carried out on demethylated apocrotonosine. With tartaric or malic acid, demethylated apocrotonosine gave characteristic fluorescein-type reactions,[7] thus showing a *meta*-orientation of the dihydric phenolic groups in ring A. The characteristic catechol colour test with ferric chloride [8] was negative. The ultraviolet spectrum ($\lambda_{max.}$ 270, 276, and 305 mμ; log ε 4·05, 4·08 and 3·8) was unchanged by addition of buffered boric acid and showed the absence of *ortho*-dihydric phenols.[8,9] Mitchell's

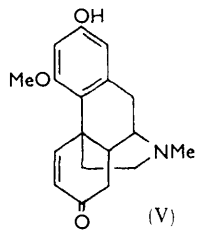
[4] Bick, Harley-Mason, Sheppard, and Vernengo, *J.*, 1961, 1896.
[5] Shoolery, "NMR and ERP Spectroscopy," Pergamon Press, Oxford, 1960.
[6] Goodwin, Shoolery, and Johnson, *Proc. Chem. Soc.*, 1958, 306.
[7] Feigl, "Qualitative Analysis by Spot Test," Elsevier Publ. Co., Amsterdam, 1946.
[8] McLean, Palmer, and Marion, *Canad. J. Chem.*, 1960, **38**, 1547.
[9] Jurd, *Arch. Biochem. Biophys.*, 1956, **63**, 376.

reagent [10] for catechols also gave a negative reaction. Crotonosine, therefore, has structure (IV; R = R′ = H).

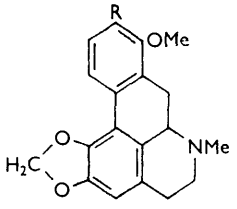
Base A, $C_{19}H_{21}NO_3$, which also has a cross-conjugated dienone grouping, is converted by acid and methylation into *NOO*-trimethylapocrotonosine methiodide. Base A is therefore *NO*-dimethylcrotonosine (IV; R = R′ = Me).

Linearisine, $C_{18}H_{21}NO_3$, absorbed 1 mol. of hydrogen in methanol in presence of 10% palladium–charcoal. With methyl iodide in the presence of potassium carbonate it gave tetrahydro-*NO*-dimethylcrotonosine methiodide. The double bond in the αβ-unsaturated carbonyl system was located by means of the ultraviolet spectrum of a methiodide of the first-stage Hofmann degradation product of linearisine; that of the linearisine methine derivative ($\lambda_{max.}$ 225, 260, and 305 mμ; log ε 4·85, 4·1, and 3·6) was similar to those of α-codeimethine and isoeugenol; but it differed from those of β-codeimethine and thebainone-β-methine [11] in lacking the intense absorption at 305 mμ. It follows that an extended chromophore is absent from the linearisine methine base and that the double bond must be located as in structure (V).

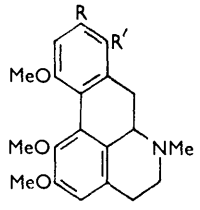
Elucidation of the structures of the major bases from *C. linearis* taken in conjunction with the proved structures of the aporphine alkaloids, crebanine (VI; R = OMe),[12]



(V)



(VI)



(VII)

stephanine (VI; R = H),[13] and the postulated structures of argemonine (VII; R = H, R′ = OMe or *vice versa*),[14] leads us to study the biogenesis of the *Croton* alkaloids.

Experimental

The general directions of the preceding paper apply here also. Rotations were for methanol solutions. Neutral alumina, graded according to the Brockmann scale of activity, was used in chromatography. Nuclear magnetic resonance spectra were kindly determined by Professor Z. Valenta (*N*-acetyl-*OO*-dimethylapocrotonosine in deuterochloroform) on a Varian Associates 60 Mc. sec.$^{-1}$ instrument and by Dr. K. Magnus (*NO*-diacetylcrotonosine and linearisine in chloroform) using a Varian Associates spectrometer model V–4300B with measurements made at 40 Mc. sec.$^{-1}$ and line positions measured by the conventional side-band technique with a Muirhead Decade oscillator (model D 695-A); in both methods tetramethylsilane was used as internal reference. Dr. Magnus also determined the spectra of *NO*-diacetyl-crotonosine and -tetrahydrocrotonosine on a Varian A-60 instrument. Line positions were determined with the aid of a semi-decade oscillator from the chloroform line (437 c./sec.).

NO-*Diacetyltetrahydrocrotonosine*.—Crotonosine (0·16 g.) was hydrogenated in ethanol with platinum as catalyst. Slightly more than 2 mol. of hydrogen were taken up in 1 hr. The tetrahydrocrotonosine was left in pyridine (15 ml.) and acetic anhydride (6 ml.) at room temperature. Working up in the usual way gave the *diacetate*, needles (from ethyl acetate), m. p. 107—108°, $[\alpha]_D^{28}$ −127·3° (*c* 1·07), $\nu_{max.}$ 3509 (OH), 1770 and 1230 (phenol OAc), 1709 (C:O) and 1639 (amide from a secondary base) cm.$^{-1}$ (Found: C, 66·2; H, 6·9; N, 3·8; O, 23·1. $C_{21}H_{25}NO_5,\frac{1}{2}H_2O$ requires C, 66·3; H, 6·9; N, 3·7; O, 23·1%).

[10] Mitchell, *Analyst*, 1923, **48**, 2.
[11] Bentley, Robinson, and Wain, *J.*, 1952, 958.
[12] Manske, " The Alkaloids," Vol. VII, Academic Press, Inc., New York, 1960, p. 429.
[13] Tomsta and Hirai, *J. Pharm. Soc. Japan* (*Yokugaka Zusshi*), 1957, **77**, 290.
[14] Shamma, *Experientia*, 1962, **18**, 64.

NO-*Dimethylcrotonosine Methiodide.*—Crotonosine (0·21 g.) was refluxed in methanol (20 ml.) in the presence of potassium carbonate (1 g.) and an excess of methyl iodide for 1 hr. The product was extracted into chloroform and worked up in the usual way, yielding crystals which when recrystallised from ethanol afforded the *methiodide* (0·15 g.), m. p. 175—177°, $\nu_{max.}$ 1667 (αβ-unsaturated C:O) and 1626 (C=C) cm.$^{-1}$ (Found: C, 52·9; H, 5·8; I, 27·4; N, 3·1; O, 10·9. $C_{20}H_{26}INO_3$ requires C, 53·0; H, 5·3; I, 27·9; N, 3·1; O, 10·6%).

NO-*Dimethylapocrotonosine Methiodide.*—Crotonosine (0·27 g.) was refluxed with 3N-hydrochloric acid (28 ml.) in methanol for 10 hr. The solution was made basic with aqueous ammonia (25% v/v) and by extraction with chloroform gave an amorphous product. This was refluxed with an excess of methyl iodide in acetone in the presence of anhydrous potassium carbonate (1 g.). The *derivative* was purified by passage in methanol through alumina (20 g.). Removal of solvent yielded crystals and recrystallisation from methanol–acetone gave cubes, m. p. 174—177°, $[\alpha]_D^{28}$ +10·7° (*c* 0·22), $\lambda_{max.}$ 269, 278, and 303 mμ (log ε 4·97, 4·98, and 4·7), ν_{max} 3279 (OH), 1613 (aromatic OMe) cm.$^{-1}$ (Found: C, 53·0; H, 5·3; I, 28·4; N, 3·2; O, 10·6; OMe, 13·6. $C_{20}H_{24}INO_3$ requires C, 53·0; H, 5·3; I, 28·0; N, 3·1; O, 10·6; 2OMe, 13·7%).

NO-*Diacetyl*-O-*methylapocrotonosine* (II; R = R′ = Ac).—The amorphous product obtained as above (0·27 g.) was treated with pyridine and acetic anhydride in the usual way. Recrystallisation of the *product* from methanol gave plates (0·20 g.), m. p. 190—191°, $[\alpha]_D^{28}$ −397° (*c* 1·2), $\lambda_{max.}$ 271, 276, and 297 mμ (log ε 4·4, 4·4, and 3·82), $\nu_{max.}$ 1757 and 1250 (phenol OAc) and 1630 (amide) cm.$^{-1}$ (Found: C, 69·4; H, 6·4; OMe, 16·3. $C_{22}H_{23}NO_5$ requires 69·3; H, 6·1; 2OMe, 16·3%). With reduced Adams catalyst, this compound showed no uptake of hydrogen after 1 hr. in ethanol at 25°/753 mm.

N-*Acetyl*-OO-*dimethylapocrotonosine* (II; R = Me, R′ = Ac).—The acid rearrangement product (0·19 g.) obtained as above was dissolved in the minimum of methanol, treated with diazomethane in ether, and left at room temperature for 24 hr. Removal of the solvent in *vacuo* gave a product which was acetylated with pyridine and acetic anhydride in the usual way. Recrystallisation of the *acetyl derivative* from methanol afforded needles, double m. p. 187—188°, 222—224°, $[\alpha]_D^{28}$ −388° (*c* 0·52), $\nu_{max.}$ 1634 (amide) and 1618 (aromatic OMe) cm.$^{-1}$ (Found: C, 71·6; H, 6·5; O, 18·3; OMe, 26·5. $C_{21}H_{23}NO_4$ requires C, 71·4; H, 6·6; O, 18·1; 3OMe, 26·3%). With reduced Adams catalyst, this compound showed no uptake of hydrogen after 1 hr. in ethanol at 27°/748 mm.

NOO-*Trimethylapocrotonosine Methiodide.*—The acid rearrangement product (0·11 g.) was methylated with diazomethane as above and the product was refluxed with an excess of methyl iodide and potassium carbonate (0·5 g.) in methanol (25 ml.). The *methiodide* recrystallised from ethanol–acetone as rods (0·09 g.), m. p. 200—201° (decomp.), $[\alpha]_D^{24}$ −70·6° (*c* 0·49), $\nu_{max.}$ 1613 (aromatic OMe) cm.$^{-1}$ (Found: C, 53·6; H, 5·5; I, 27·3; N, 2·9; O, 10·7; OMe, 19·0. $C_{21}H_{26}INO_3$ requires C, 53·9; H, 5·6; I, 27·2; N, 3·0; O, 10·3; 3OMe, 19·9%).

Conversion of Base A into NOO-*Trimethylapocrotonosine Methiodide.*—Base A (0·06 g.) was converted into a rearrangement product under similar conditions to those used for crotonosine. The product was methylated and converted into a methiodide (0·03 g.) as above. Recrystallisation from methanol afforded rods, m. p. 200—201° (decomp.) alone or mixed with *NOO*-trimethylapocrotonosine methiodide which had the same infrared spectrum.

Tetrahydro-NO-*dimethylcrotonosine Methiodide.*—(*a*) Tetrahydrocrotonosine (0·17 g.) was refluxed with an excess of methyl iodide and potassium carbonate (0·15 g.) in methanol (25 ml.) for 1—2 hr. Solvent was then removed in *vacuo* and the product extracted in chloroform. Removal of the chloroform *in vacuo* and addition of a few drops of acetone–methanol–benzene afforded the *product* that, recrystallised from acetone–methanol, had m. p. 258° (decomp.), (0·12 g.), $[\alpha]_D^{24}$ +0·64° (*c* 0·63), $\nu_{max.}$ 1613 (aromatic OMe) cm.$^{-1}$ (Found: C, 52·4; H, 6·3; I, 27·5; N, 3·0. $C_{20}H_{28}INO_3$ requires C, 52·5; H, 6·2; I, 27·7; N, 3·1%).

(*b*) Linearisine (0·10 g.) was hydrogenated in methanol over reduced 10% palladised charcoal (100 mg.) (uptake 0·85 mol. in 1 hr.). The product was converted into the *O*-methyl methiodide as above. Recrystallisation from methanol afforded cubes, 0·08 g., m. p. 258° (decomp.), identified with tetrahydro-*NO*-dimethylcrotonosine methiodide as usual.

Hofmann Degradation.—Linearisine (0·03 g.) was refluxed with an excess of methyl iodide in methanol (12 ml.) for 1 hr. Solvent and reagent were removed in *vacuo*, 5% aqueous sodium hydroxide (10 ml.) was added, and heating continued for 1 hr. at 120°. The solution was made acidic with 3N-hydrochloric acid and then adjusted to pH ~9 with aqueous ammonia. An oil was obtained from a chloroform extract and was converted into the quaternary salt by methyl

iodide in boiling methanol (8 ml.). Removal of solvent yielded crystals and recrystallisation from acetone–ethanol gave material of m. p. 132—135°, $\nu_{max.}$ 3226 (OH), 1667 (αβ-unsaturated C:O), 1626 (C=C) cm.$^{-1}$.

Acetolysis of NO-*Dimethylcrotonosine Methiodide.*—The method was essentially that used by Schryner and Lees.[3] *NO*-Dimethylcrotonosine methiodide (0·5 g.), acetic anhydride (10 g.), and anhydrous sodium acetate (0·5 g.) were heated until all the solids had dissolved. Silver acetate (0·8 g.) was added, and heating was continued at 160° for 6 hr. The green solution produced was cooled and filtered. The filtrate was heated at 180° for 3 hr. in a sealed Pyrex tube. Extraction by chloroform (5 × 50 ml.) yielded a brown oil which was extracted once with boiling 10 : 1 v/v light petroleum (b. p. 60—80°)–acetone (150 ml.). The solvents were removed from the extract in *vacuo* and the product was chromatographed on alumina (grade 3). Elution with chloroform afforded a yellow oil (0·16 g.) which on sublimation at 150—190°/1 mm. gave a pale yellow nitrogen-free solid which softened at 130° and had $\lambda_{max.}$ 257, 307, 345, and 365 mμ (log ε ~4·9, 3·7, 2·1, and 1·9) with shoulders at ~265, 280, and 315 mμ (log ε 4·6, 3·8, and 3·6), $\lambda_{min.}$ 225, 290, and 355 mμ (log ε ~4·2, 3·4, and 1·7), $\nu_{max.}$ (in $CHCl_3$) 1754 and 1250 (phenol OAc) cm.$^{-1}$.

Demethylapocrotonosine.—Crotonosine 0·10 g. was heated with glacial acetic acid (10 ml.) and hydriodic acid (3 ml.) at 145° for 1 hr. After removal of solvent and reagent *in vacuo*, water was added (15 ml.) and the solution adjusted to ~pH 8 with aqueous ammonia. The base was extracted in chloroform. After removal of the chloroform, a few drops of ethanol were added, affording crystals (0·03 g.) of *demethylapocrotonosine*, which, recrystallised from acetone–ethanol, had m. p. 200—202°, $\lambda_{max.}$ 270, 276, and 305 mμ (log ε 4·05, 4·08, and 3·8), $\nu_{max.}$ 3340 (OH) cm.$^{-1}$ (Found: C, 71·5; H, 5·7. $C_{16}H_{15}NO_3$ requires C, 71·4; H, 5·6%).

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