334. *Alkaloids from* Croton *species. Part I. The Isolation of Alkaloids from* C. linearis *Jacq., and the Detection of Alkaloids in* C. glabellus *L.*, C. humilis *L., and* C. flavens *L.*

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The isolation and characterisation of four new alkaloids from *C. linearis* Jacq. is described. Presence of alkaloids (unidentified) in the other species named in the title is indicated qualitatively.

As part of a general programme directed to the evaluation of plants used in Jamaican folk medicine, a chemical and preliminary pharmacological examination has been made of *Croton linearis* Jacq. (" Spanish rosemary ").

C. linearis Jacq. which is used in Jamaica in the treatment of fevers, colds, and colic,[1] yielded alkaloidal material by two main extraction techniques, (*a*) methanolic extraction of the dried plant and (*b*) percolation by 2% tartaric acid solution. The annexed Table

Method and wt. of dried plant material *	Total yield (%)	Counter-current distribution fractions, as yields (% of dried plant)					
		A	B	C	C_1	E	F
MeOH method: 1·4 kg.	1	0·19 (0·009 †)	0·09	0·028 †	—	0·23	0·18 †
Tartaric acid method: 3·5 kg. ...	0·6	0·1	0·075	0·003 †	0·012 †	0·167	0·059 †

* 80% of fresh weight is lost on drying. † Crystalline material.

shows a comparison of these two methods. The chloroform-soluble bases were separated by countercurrent-distribution, the separation being followed by descending paper

[1] Asprey and Thornton, *W.I. Med. J.*, 1955, **4**, 78.

chromatography: in some instances further purification by column chromatography was necessary before crystalline bases were obtained. The separated bases were temporarily designated A, B, C, C_1, E, and F. Method (*b*) is more economical and more easily manipulated, and also extracts base C_1 which was not obtained by the former method. The yield of 0·6% shown in the Table for the tartaric acid percolation was subsequently improved to 0·85% by using larger volumes of acid solution, but the percentage distribution of individual bases has not yet been determined for this yield.

Base A, $C_{19}H_{21}NO_3$, m. p. 127—128°, $[\alpha]_D^{24}$ +111·2° (*c* 1·24 in EtOH), pK_a 6·2, was obtained by purification of fraction A. It contained two methoxyl groups, one *N*-methyl group, and no *C*-methyl or phenolic group. Its ultraviolet spectrum was similar to those of the other crystalline bases, and infrared absorption at 1658 cm.$^{-1}$ indicates a cross-conjugated dienone system.[2] The methiodide had m. p. 244—245° (decomp.).

Base B gave one spot on one-dimensional paper chromatograms but was resolved into two amorphous fractions when run on an aluminium oxide column. Acetylation of one fraction (BIa) gave a crystalline derivative, m. p. 210°.

Base C, $C_{19}H_{23}NO_3$, named homolinearisine, seemed to suffer marked seasonal variation in yield. When recrystallised from ethanol it formed anisotropic plates, m. p. 220—223° (decomp.), $[\alpha]_D^{29}$ +84·4° (*c* 0·84 in MeOH), pK_a 6·6. It contained one methoxyl and one *N*-methyl group, but no *C*-methyl group. Infrared absorption indicated an αβ-unsaturated carbonyl group (1664 cm.$^{-1}$), unsaturation (1625 cm.$^{-1}$), a phenol (1210 cm.$^{-1}$), a possible tertiary nitrogen (1347 cm.$^{-1}$), and substitution on an aromatic ring (865, 695 cm.$^{-1}$), and there were aromatic C=C in-plane vibration bands at 1600 and 1500 cm.$^{-1}$. The hydrochloride did not melt below 300°; the perchlorate had m. p. 184—186°.

Base C_1, $C_{18}H_{21}NO_3$, which was named linearisine, formed anisotropic rods, m. p. 219—222°, $[\alpha]_D^{28}$ +116° (*c* 0·83 in MeOH), pK_a 6·4, and contained one methoxyl and one *N*-methyl but no *C*-methyl group. The infrared spectrum was similar to that of homolinearisine except for the absence of a band at 850 cm.$^{-1}$ in linearisine; there was increased intensity of absorption at 918 cm.$^{-1}$ and a weak band at 685 cm.$^{-1}$. The absorption band indicating unsaturation was shifted from 1625 cm.$^{-1}$ in homolinearisine to 1608 cm.$^{-1}$ in linearisine.

Base E was found in the same tubes as base F in countercurrent experiments with the system chloroform–sodium acetate buffer (pH 5·59). When a few drops of methanol were added to a solid mixture of materials E and F, base F crystallised. Base E was also separated from F by taking advantage of the fact that base F did not form a precipitate under the usual conditions specified for Mayer's test. The Mayer precipitate of base E was treated with hydrogen sulphide and extracted from a basified solution; a small amount of crystalline product, m. p. 174—178°, was obtained from a solution of this extracted material in methanol–acetone mixture by slow evaporation.

Base F, $C_{17}H_{17}NO_3$, $[\alpha]_D^{28}$ +180° (*c* 0·83 in MeOH), pK_a 7·3, was called crotonosine, and is the major crystalline alkaloidal component of *C. linearis*. When recrystallised from propan-2-ol, it began to decompose at 165°, softened at 197°, but did not melt below 300°. It contained one methoxyl group but no *N*- or *C*-methyl group. The phloroglucinol–concentrated sulphuric acid test for methylenedioxy-groups was negative, as it was also for base A, homolinearisine, and linearisine. The *NO*-diacetyl derivative had m. p. 203—205°. Crotonosine gave a precipitate with Mayer's reagent in 3N-nitric acid and 6N-sulphuric acid, but not in 1—3N-hydrochloric acid. Crotonosine hydrochloride did, however, give a Mayer's precipitate when dissolved in water and apparently the HgI_3 complex formed in the case of Mayer's precipitates[3] was very soluble in the presence of an excess of chloride ions, especially when the proton concentration was increased above a critical level.

[2] Barton and Scott, *J.*, 1958, 1767.
[3] Levi and Farmilo, *Analyt. Chem.*, 1954, **26**, 1040.

Sparsiflorine, $C_{17}H_{17}NO_3$, isolated from *C. sparsiflorus* Morong,[4] is not identical with crotonosine. The infrared spectrum of crotonosine showed a strong band at 3220 cm.$^{-1}$, absent from the spectrum of the *NO*-diacetyl derivative and due to a secondary amino-group. The presence of a phenolic hydroxyl group was indicated by a ferric chloride and a Millon reaction and by a broad absorption band at 2600 cm.$^{-1}$ (bonded OH) along with bands at 1775 and 1258 cm.$^{-1}$ (phenolic OAc) in the spectrum of *NO*-diacetylcrotonosine. This evidence was supported by a bathochromic shift of the ultraviolet spectrum on addition of dilute sodium hydroxide. The infrared spectrum of crotonosine also had a band at 1664 cm.$^{-1}$ which, in conjunction with that at 235 mμ in the ultraviolet region, was interpreted as due to a cross-conjugated dienone.[2] Absorption at 1622 cm.$^{-1}$ was due to unsaturation and this assignment was supported by its disappearance on hydrogenation in which 2 mol. of hydrogen were taken up.

The hydrogenated material had a carbonyl band at 1706 cm.$^{-1}$, shifted from 1664 cm.$^{-1}$ for crotonosine. A very strong band at 858 cm.$^{-1}$ was probably due to the unsaturation (absorption reduced on hydrogenation) and to substitution in the aromatic ring.[5]

Later an alkaloidal screening indicated the presence of alkaloids also in *C. flavens* L., *C. glabellus* L., and *C. humilis* L. The method consisted of digesting previously dried plant material with N-hydrochloric acid on a water-bath, basification with ammonia, and extraction with chloroform. The crude material extracted was tested with Mayer's reagent and also by a colour reaction on paper with platinum chloride–potassium iodide spray.

Experimental

M. p.s were determined on a Kofler block. Ultraviolet spectra were determined on a Beckman model DU spectrophotometer for ethanol solutions, and infrared spectra for Nujol mulls in a Perkin-Elmer model 21 spectrophotometer. pK_a determinations were in 60% ethanol and 0·1N-hydrochloric acid was used for titrations.

Methanolic Extraction.—Dried, powdered plant material (1·4 kg.) was extracted continuously with methanol for 48 hr. in a large Soxhlet-type extractor, and the solution concentrated *in vacuo* to a thick black residue which was digested with N-hydrochloric acid. A small-scale ether extraction of this acid solution showed that only a very small amount of acidic or neutral non-alkaloidal components was present, so a continuous extraction with chloroform was carried out after basification with ammonia solution. A brown crude alkaloidal mixture (15·3 g.) was obtained on removal of the chloroform.

Crude material (7·5 g.) from the methanolic extraction was extracted in a countercurrent apparatus with chloroform and 0·2N-acetate buffer (pH 5·59) as the moving phase, corresponding to a partition ratio of 1·33 at 19—21°. A 60 tube (40 ml.) transfer was carried out with shaking time 1½ min., and settling time 2 min.

Base A. Tubes 1—3 yielded 1·43 g. of brown amorphous material. Impure base A (4·2 g.) from three countercurrent separations was digested with N-hydrochloric acid, and basic material was reprecipitated with aqueous ammonia. This material was extracted with chloroform, and the base so obtained passed through an alumina column packed in methanol. Unabsorbed material was extracted with light petroleum (b. p. 60—80°), and material so obtained was dried over silica gel. Small amounts of ether were then added, and the flask scratched, to afford *base A* (0·32 g.), m. p. 127—128°, $\lambda_{max.}$ 227, 230, 280, and 285 mμ (log ε 4·40, 4·41, 3·55, and 3·55) $\nu_{max.}$ 1658 (C:O in cross-conjugated dienone), 1618 (aromatic OMe), 1605, and 1486 (aromatic C=C in-plane vibration) cm.$^{-1}$ (Found: C, 73·4; H, 7·1; N, 4·4; O, 15·4; *N*-Me, 4·9; OMe, 20·2%; *M*, 317; *C*-Me, 0. $C_{19}H_{21}NO_3$ requires C, 73·3; H, 6·8; N, 4·5; O, 15·4; 1*N*-Me, 4·8; 2OMe, 19·9%; *M*, 311·4).

Base A (0·05 g.) was refluxed with potassium carbonate (0·5 g.) and excess of methyl iodide in methanol. Solvent was removed *in vacuo* and the *methiodide* extracted in chloroform. Evaporation and addition of a few drops of methanol gave crystals. Recrystallisation from ethanol afforded needles, m. p. 244—245° (decomp.), $\nu_{max.}$ 1656 (cross-conjugated dienone,

[4] Saha, *Science and Culture*, 1959, **24**, 572.
[5] Bellamy, "The Infra-red Spectra of Complex Molecules," London, Methuen and Co. Ltd., 2nd edn.

C:O) cm.$^{-1}$ (Found: C, 53·0; H, 5·4; I, 28·3; N, 3·3; O, 10·4; *N*-Me, 6·6; OMe, 13·8. $C_{20}H_{24}INO_3$ requires C, 53·0; H, 5·3; I, 28·0; N, 3·1; O, 10·5; 2*N*-Me, 6·6; 2OMe, 13·7%).

Base B. Tubes 4—23 yielded amorphous material (0·73 g.). Crude base (0·27 g.) was run on an alumina column [Brockmann's grade 3 in ethyl acetate–benzene–methanol (3 : 3 : 1 v/v)]. Fraction 1 (0·17 g.) was eluted with ethyl acetate as a yellow band, and fraction 2 (0·08 g.) with methanol. Both fractions gave two spots on Whatman no. 4 paper developed with ethyl acetate–pyridine–water (7·5 : 2·3 : 1·65 v/v). Fraction 1 was treated with acetic anhydride in pyridine and the product separated on a short alumina column in methanol. Of the resulting fractions, BIa gave a positive platinum chloride reaction and BIb did not. The latter result can be interpreted as due to a neutral derivative or non-alkaloidal material. Fraction BIa yielded crystals (0·04 g.), m. p. 210°, and since it formed no *N*-acetyl derivative it possibly contains a tertiary nitrogen.

Homolinearisine (*base C*). This *base* occurred in tubes 24—39 (0·20 g.); recrystallisation from ethanol afforded plates, m. p. 220—223° (decomp.), $\lambda_{max.}$ 228, 282, and 288 mμ (log ε 4·29, 3·18, and 3·19), $\nu_{max.}$ 1664 (αβ-unsaturated C:O) and 1625 (C=C) cm.$^{-1}$ (Found: C, 72·8; H, 7·4; N, 4·6; O, 15·3; *N*-Me, 4·7; OMe, 9·6%; *M*, 318; *C*-Me, 0. $C_{19}H_{23}NO_3$ requires C, 72·8; H, 7·4; N, 4·5; O, 15·3; 1*N*-Me, 4·8; 1OMe, 9·9%; *M*, 313·4). Homolinearisine gave with ferric chloride in ethanol and with potassium dichromate–concentrated sulphuric acid a green-brown colour, with Fröhde's reagent a purple colour becoming grey and then green, and a white precipitate with Mayer's reagent.

Homolinearisine was dissolved in the minimum of methanol, concentrated hydrochloric acid added dropwise until the solution was acidic, then the solvent was allowed to evaporate in a desiccator. Crystals were collected. Recrystallisation from ether–acetone–ethanol afforded the *hydrochloride* as rods, not melting below 300°, with $\nu_{max.}$ 2570—2420s (NH^+) and 1668 (αβ-unsaturated C:O) (Found: C, 65·3; H, 6·8; Cl, 10·5; N, 3·9; O, 14·2. $C_{19}H_{23}NO_3$,HCl requires C, 65·2; H, 6·9; Cl, 10·1; N, 4·0; O, 13·7%). The *perchlorate* was prepared by dissolving homolinearisine (40 mg.) in the minimum of ethanol, adding five drops of 80% perchloric acid, and then ether dropwise. The precipitate yielded needles (from ethanol–ether), m. p. 184—186°, $\nu_{max.}$ 3480 (OH), 1660 (αβ-unsaturated C:O), and 1157—1025sbr cm.$^{-1}$ (ClO_4^-) (Found: C, 53·8; H, 5·5; Cl, 9·2; N, 3·6; O, 28·0. $C_{19}H_{23}NO_3$,$HClO_4$,$\frac{1}{2}H_2O$ requires C, 53·8; H, 5·7; Cl, 8·4; N, 3·3; O, 28·3%).

Base E. Tubes 40—60 yielded a mixture (2·0 g.) of bases E and F, from which the latter crystallised on addition of methanol. From the amorphous residue (1·67 g.) in N-hydrochloric acid, base E was precipitated with Mayer's reagent; the filtrate was basified with aqueous ammonia and base F removed therefrom in chloroform. Base E was regenerated by resuspending the precipitate in N-hydrochloric acid and bubbling in hydrogen sulphide. After filtration and basification with aqueous ammonia, base E was extracted in chloroform. Material obtained on removal of the solvent *in vacuo* was dissolved in methanol–acetone (1 : 1 v/v), and crystals (10 mg.), m. p. 174—178°, were obtained on slow evaporation at room temperature.

Crotonosine (*base F*). This *base* (0·32 g.) separated from a 2·0 g. mixture of bases E and F (tubes 40—60) (see above). It recrystallised from propan-2-ol and softened at 197° with decomposition but did not melt below 300°; it had $\lambda_{max.}$ 226, 235, 282, and 290 mμ (log ε 4·30, 4·33, 3·37, and 3·41), $\nu_{max.}$ 3220 (NH), 2600 (bonded OH), 1664 (C:O in cross-conjugated dienone), 1622 (C=C), and 858vs (C=C and aromatic substitution) cm.$^{-1}$ (Found: C, 72·0; H, 6·1; N, 4·9; O, 17·0; OMe, 11·3; *M*, 274; *N*-Me and *C*-Me, 0. $C_{17}H_{17}NO_3$ requires C, 72·1; H, 6·1; N, 4·9; O, 16·9; OMe, 10·8%; *M*, 283·3). It gave a brown colour, becoming green, with potassium dichromate–concentrated sulphuric acid, with Fröhde's reagent a light blue colour becoming dark green, and with ethanolic ferric chloride an orange colour.

Crotonosine (0·10 g.) and methyl iodide in warm acetone–methanol gave a *methiodide* which from ethanol then methanol formed rods, decomp. >250°, $\nu_{max.}$ 3333 (OH) and 1667 (cross-conjugated dienone C:O) cm.$^{-1}$ (Found: C, 48·9; H, 4·6; N, 3·6; *N*-Me, 3·8. $C_{18}H_{20}INO_3$ requires C, 48·8; H, 4·5; N, 3·2; 1*N*-Me, 3·3%).

Crotonosine (25 mg.) in pyridine (2·5 ml.) and acetic anhydride (1·5 ml.) were left at room temperature for 15 hr., then evaporated *in vacuo*, and water was added to turbidity. Crystals separated at 0°. Recrystallisation from ethyl acetate yielded the NO-*diacetyl derivative* as cubes (16 mg.), m. p. 203—205°, $\nu_{max.}$ 1775 and 1258 (aromatic OAc), 1670 (cross-conjugated dienone C:O), 1640 (tertiary amide), and 1628 (C=C) cm.$^{-1}$ (Found: C, 68·8; H, 5·9; N, 3·7; O, 22; Ac, 22·9. $C_{21}H_{21}NO_5$ requires C, 68·9; H, 5·8; N, 3·8; O, 21·8; 2Ac, 23·4%).

Tartaric Acid Extraction.—2% Tartaric acid solution (28 l.) was percolated through a 20 l. aspirator packed with dried plant material (3·5 kg.). The extract was concentrated in a cyclic evaporator at 40° to 1·7 l. Precipitated tartaric acid and non-alkaloidal salts were filtered off. Small quantities of 0·5N-hydrochloric acid were used to remove base adsorbed on the solid. The combined solutions were basified with aqueous ammonia solution, and bases were extracted with chloroform. The chloroform was removed *in vacuo*, a small volume of methanol added, and the brown syrup left overnight. Crotonosine (0·97 g.) crystallised and was filtered off after acetone had been added to loosen crystals from the syrup. The filtrate, when dried, yielded a crude mixture (20 g.) of bases, including additional amounts of crotonosine. Countercurrent distribution of this material (10 g.) under the conditions described above gave: fraction A, tubes 1—4 (1·86 g.); fraction B, tubes 5—19 (1·31 g.); homolinearisine (base C), tubes 21—25 (0·06 g.); linearisine (base C_1), tubes 26—31, (0·21 g.); crotonosine (base F), tubes 32—60 (1·04 g.); and 2·95 g. of base E–F mixture.

Linearisine. Crystals obtained from tubes 26—31 recrystallised from ethanol as anisotropic rods, m. p. 219—222° (decomp.), $\lambda_{max.}$ 228, 282, and 288 mμ (log ε 4·30, 3·19, and 3·22), $\nu_{max.}$ 1664 (αβ-unsaturated C:O), 1608 (C=C), 1600, and 1500 (aromatic C=C in-plane vibration) and 865, 685 (aromatic substn.) cm.$^{-1}$ (Found: C, 72·1; H, 7·1; N, 4·5; O, 15·9; *N*-Me, 4·7; OMe, 9·9%; *M*, 345. $C_{18}H_{21}NO_3$ requires C, 72·2; H, 7·1; N, 4·7; O, 16·0; 1*N*-Me, 5·0; 1OMe, 10·4%; *M*, 299·4).

This *linearisine* was treated as in the preparation of homolinearisine hydrochloride. The *hydrochloride* obtained recrystallised from ethanol as rods, decomp. <300°, $\nu_{max.}$ 2632—2353 (NH^+) and 1681 (αβ-unsaturated C:O) cm.$^{-1}$ (Found: C, 64·6; H, 6·4; Cl, 10·5; N, 4·0; O, 14·3. $C_{18}H_{21}NO_3$,HCl requires C, 64·7; H, 6·6; Cl, 10·6; N, 4·2; O, 14·3%).

Paper Chromatography.—The system ethyl acetate–pyridine–water (7·5 : 2·3 : 1·65 v/v) was used with Whatman no. 4 paper for descending chromatography in a sealed tank. Solution samples from alternate tubes of the countercurrent distribution were spotted on the paper and the bases converted into the hydrochlorides by 0·1N-hydrochloric acid in ethanol. The papers were developed for 5 hr. and then air-dried. Platinum chloride–potassium iodide spray was used to detect alkaloids. All spots were violet, except those of crotonosine and pyridine hydrochloride which were blue-green. The following R_F values were obtained at 25—26° in a typical run. Base A, 0·82; base B, unseparated 0·58; homolinearisine (C), 0·75; linearisine (C_1), 0·65; crotonosine (F) (2 spots), 0·53, 0·47; base E, 0·57; pyridine hydrochloride, 0·18—0·20.

Test for the Homogeneity of Crotonosine.—Crotonosine (5 mg.) in ethanol was put on Whatman no. 4 paper along a line 2″ from the paper edge and developed in ethyl acetate–pyridine–water (7·5; 2·3 : 1·65 v/v) at 25° for 8 hr. The paper was then air-dried. The base gave two bands, A and B, which were cut out with the aid of guide strips. The bands were each extracted with ethanol–chloroform and rechromatographed after concentration of the extract to a few drops; for each band extracted two spots again developed at the above stated R_F values for crotonosine.

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