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ALKALOID BIOSYNTHESIS IN *CROTON LINEARIS**

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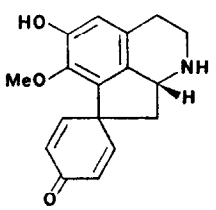
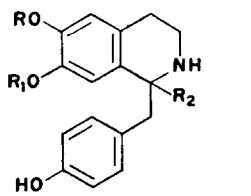
Key Word Index—*Croton linearis*, Euphorbiaceae, proaporphine alkaloid, crotonosine, linearisine, biosynthesis

Abstract—The ability of *Croton linearis* to form crotonosine from linearisine has been demonstrated, the extent of this conversion appears to vary according to the sex of the plant

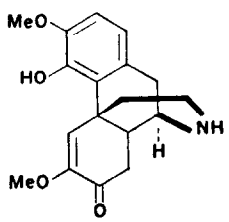
EARLIER biosynthetic studies on *Croton linearis*[1] demonstrated that crotonosine (I) was formed from (+)-coclaurine (II) or (±)-norcoclaurine (III), and that 8,14-dehydronorsalutaridine (IV) could be biosynthesized from (±)-coclaurine, (±)-norcoclaurine and (±)-isococlaurine (V)[2] in this plant There has been some speculation as to the relationship



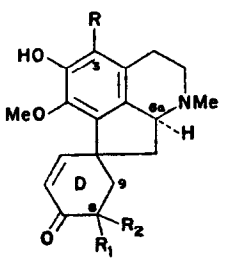
(I)



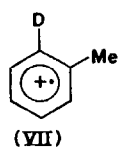
(II) R = Me, R_1 = H, R_2 = ⇀H
(III) R = R_1 = H, R_2 = ⇁H
(V) R = H, R_1 = Me, R_2 = ⇁H



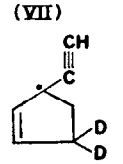
(IV)



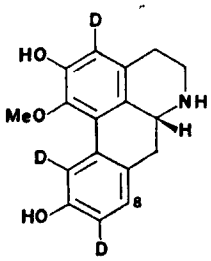
(VI) R = R_1 = R_2 = ^{3}H
(IX) R = R_1 = R_2 = ^{2}H



(VII)



(VIII)



(X)

between reduced proaporphines and proaporphines and more recently we demonstrated that crotonosine could be elaborated from $^3H_3$-linearisine (VI).[3] On repeating this, we discovered that incorporation results were consistently different for female plants than for

* Part XIV in the series "Alkaloids from *Croton* species" For Part XIII see STUART, K L and GRAHAM, L (1973) *Phytochemistry* **12**, 1967

[1] BARTON, D H R, BHAKUNI, D S, CHAPMAN, G M, KIRBY, G W, HAYNES, L J. and STUART, K L. (1967) *J Chem Soc* C, 1295.
[2] HAYNES, L J, HUSBANDS, G E M and STUART, K L (1968) *J Chem Soc* C, 951
[3] STUART, K L and GRAHAM, L (1971) *Chem Commun* 392

male plants For female plants we observed incorporation values of 0 01–0 02% while in male plants it ranged from no incorporation to 0 002% incorporation Although these experiments may indicate some differences of the biosynthesizing enzymes between the sexes, other factors such as differences in the age of the plants may have had some influence [4] The labelling pattern of $^3H_3$-linearisine was determined by doing parallel experiments on linearisine but using D_2O rather than T_2O Confirmation of the location of the deuterium was by NMR and MS examination Comparison of the NMR spectrum of non-deuterated linearisine and deuterated linearisine showed that the deuterated sample, obtained by heating the alkaloid under nitrogen in dimethylformamide with D_2O had the C-3 proton (δ 6 43) and two aliphatic protons (δ 2 4–3 5) missing [5] The MS comparison is shown in Table 1 and the interpretation is based on earlier studies on this and related alkaloids [6] A fragment in the spectrum of a deuterated sample was established by high resolution MS to have a *m/e* of 93 0687 and could be due to either of the deuterated fragments (VII) or (VIII), for which the calculated values are 93 0708 and 93 0647 respectively All the foregoing evidence is consistent with structure (IX) and this labelling pattern, especially with regards to the specific replacement of the α-methylene protons in ring D of linearisine is similar to that observed for testosterone, in which case enolization occurred almost exclusively toward C-2 [7]

TABLE 1 MS COMPARISON OF NON-DEUTERATED AND DEUTERATED LINEARSINE

Type ion	Non-deuterated *m/e*	Non-deuterated % Base peak	Deuterated *m/e*	Deuterated % Base peak
M^+	299	90	302	59
(P + 1)	300	46 5	303	23
(P − 1)	298	89	301	100
(P − Me)	284	18	287	10
(P − OMe)	268	5	271	5
(P − NC_2H_5)	256	100	259	20

Because the utilization linearisine for the elaboration of crotonosine either involved the intermediacy of an imine or enamine, it was important to establish that in the deuterated compound no racemization had occurred during the conversion This was done by showing that the specific rotation of the deuterated compound was the same as that for the non-deuterated linearisine ($[\alpha]_D$ +117 5°)

In an attempt to establish the location of the tritium atoms in the isolated crotonosine, the acid rearranged product, apocrotonosine (X), was deuterated This resulted in the removal of 88% of the radioactivity If the activity was equally distributed between two labelled positions, then this represents a 94% exchange at each tritiated site

The conversion of linearisine (VI) to crotonosine (I) must involve *N*-demethylation, inversion of the asymmetric centre at C-6a as well as oxidation of carbons 8 and 9 in ring D

[4] SPENSER, I D (1968) *Comprehensive Biochemistry* (FLORKIN, M and STOTZ, E H , eds), p 237, Elsevier, New York
[5] GRAHAM, L (1972) Ph D Thesis, University of the West Indies, p 113 for spectra
[6] BALDWIN, M , LOUDON, A G , MACOLL, A , HAYNES, L J and STUART, K L (1967) *J Chem Soc* **C**, 154
[7] MALHOTRA, S K and RINGOLD, H J (1964) *J Am Chem Soc* **86**, 1997

It has been assumed in the past that reduced proaporphines are derived from proaporphines, but these experiments clearly indicate that the reverse process can also occur

EXPERIMENTAL

Deuteration and tritiation of linearisine Linearisine (90 mg) which was isolated from *Croton linearis* Jacq using the technique earlier published[8] was dissolved in dimethylformamide (2 ml), D_2O (2 ml) added, and the solution sealed under N_2 in a thick glass tube The tube was heated at 100° (72 hr) The mixture was then cooled to room temp chilled in ice and opened After evaporation under reduced pressure, the solution was purified on a short alumina column with $CHCl_3$ Evaporation of the $CHCl_3$ yielded linearisine (31 5 mg) Tritiation was achieved in a similar manner when T_2O was used instead of D_2O, and material obtained in several experiments ranged from 20 0 to 56 1 μCi

Feeding of $^3H_3$-linearisine and extraction of crotonosine Male plants and female plants were fed in separate experiments by the wick feeding technique, and in a typical feeding, the fed branch (130–250 g) was milled and extracted with 2% tartaric acid [8] After basification, continuous extraction with $CHCl_3$, removal of the solvent and addition of a few drops of acetone yielded crotonosine Radioisotopic purity was achieved by preparing the NO-diacetyl derivative [8] Male plants showed incorporation values varying from no incorporation to 0 002% while female plants had incorporation values of 0 01–0 02%

Preparation of deuterated apocrotonosine This experiment was performed in an attempt to locate all the tritium atoms in crotonosine Radioactive crotonosine (30 mg, activity = $7\ 5 \times 10^{-3}$ μCi based on the diacetate) was dissolved in 6 N HCl (1 5 ml) and heated at 100° for 1 hr [9] The resulting apocrotonosine ($7\ 4 \times 10^{-3}$ μCi) was heated at 100° (12 hr) with K *t*-butoxide (30 mg) and D_2O (1 ml) under N_2 in a sealed tube The NMR of the product was consistent with structure (X), but scintillation counting carried out as outlined in Part XIII[10] still showed some residual activity ($8\ 88 \times 10^{-4}$ μCi or 12%)

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[8] HAYNES, L J and STUART, K. L (1963) *J Chem Soc* 1784
[9] BARTON, D H R , BHAKUNI, D S , CHAPMAN, G M , KIRBY, G. W , HAYNES, L J and STUART, K L (1967) *J Chem Soc* C, 1265
[10] STUART, K L and GRAHAM, L (1973) *Phytochemistry* **12**, 1967