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ALKALOID BIOSYNTHESIS IN *CROTON FLAVENS**

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Key Word Index—*Croton flavens*, Euphorbiaceae, morphinandienone alkaloids, norsinoacutine, sinoacutine, flavinantine, biosynthesis

Abstract—The biosynthesis of the morphinandienone alkaloids norsinoacutine, sinoacutine and flavinantine has been studied using 1-^{3}H-sinoacutine, 1-^{3}H-norsinoacutine, 1-^{3}H-norsinoacutinols, L-[*S*-methyl-^{14}C]-methionine, glycine-2-^{14}C, 1-^{3}H-8,14-dihydronorsalutaridine, 1-^{3}H-8,14-dihydrosalutaridine, 1-^{3}H-sinomenine, 1-^{3}H-isosinomenine, (±)-[2-^{14}C]phenylalanine, (±)-[*N*-methyl-^{14}C]orientaline and (±)-[*N*-methyl-^{14}C]reticuline

A PRELIMINARY report[1] showed that (±)-[*N*-methyl-^{14}C]reticuline (I) was 10 times more efficiently incorporated into flavinantine (II) in *Croton flavens* than the isomeric compound (±)-[*N*-methyl-^{14}C]orientaline (III) Whereas (±)-reticuline is most probably oxidized first to isosalutaridine (IV), (±)-orientaline was probably utilized via the bisdienone (V), and then by rearrangement formed flavinantine directly, but by a minor pathway Although the formation of flavinantine from (±)-reticuline seems to involve demethylation and remethylation after the initial oxidation involving a *para-para* diradical coupling, the fact that degradation[2] of radioactive flavinantine gave an inactive phenanthrene (VI), m p 186–188°, indicates that the *N*-methyl group of flavinantine is in no way involved in the remethylation process even if flavinantine and flavinine (VII), a known component of *C. flavens*,[3] are interconvertible Such interconvertibility has been shown for sinoacutine (VIII) and norsinoacutine (IX)

Degradation of flavinantine [activity 1·0] to the phenanthrene (VI) [activity 0·47] after feeding experiments with (±)-[2-^{14}C]phenylalanine showed that for flavinantine, as for the morphine alkaloids, two molecules of this amino acid were utilized by equivalent pathways to form one molecule of this alkaloid. Other wick-feeding experiments clearly demonstrated the interconvertibility of sinoacutine (VIII) and norsinoacutine (IX) Also, the 1-^{3}H-norsinoacutinols (X) were utilized by the plant to form norsinoacutine and sinoacutine.[4] This latter experiment clearly indicates that, unlike *Papaver somniferum* plants which can dehydrate 1-^{3}H-salutaridinol (XI) to form thebaine (XII), *C flavens* as well as *Sinomenium acutum*[5] plants which contain morphinandienone compounds but do not produce morphine-type alkaloids, are able to suppress the necessary dehydration step by oxidizing dienols to

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* Part XIII in the series "Alkaloids from *Croton* species" For Part XII see KUTNEY, J P, KLEIN, F. K, EIGENDORF, G., MCNEILL, D and STUART, K L (1971) *Tetrahedron Letters* 4973, (1972) *ibid* 250.

[1] STUART, K. L., TEETZ, V. and FRANCK, B (1969) *Chem Commun* 33

[2] DOPKE, W, FLENTJE, H and JEFFS, P W (1968) *Tetrahedron* **24**, 4459.

[3] STUART, K. L. (1971) *Chem Rev* **71**, 47

[4] STUART, K L. and GRAHAM, L (1971) *Chem. Commun* 392

[5] BARTON, D H R, KIRBY, A J and KIRBY, G W (1968) *J Chem Soc* C, 929

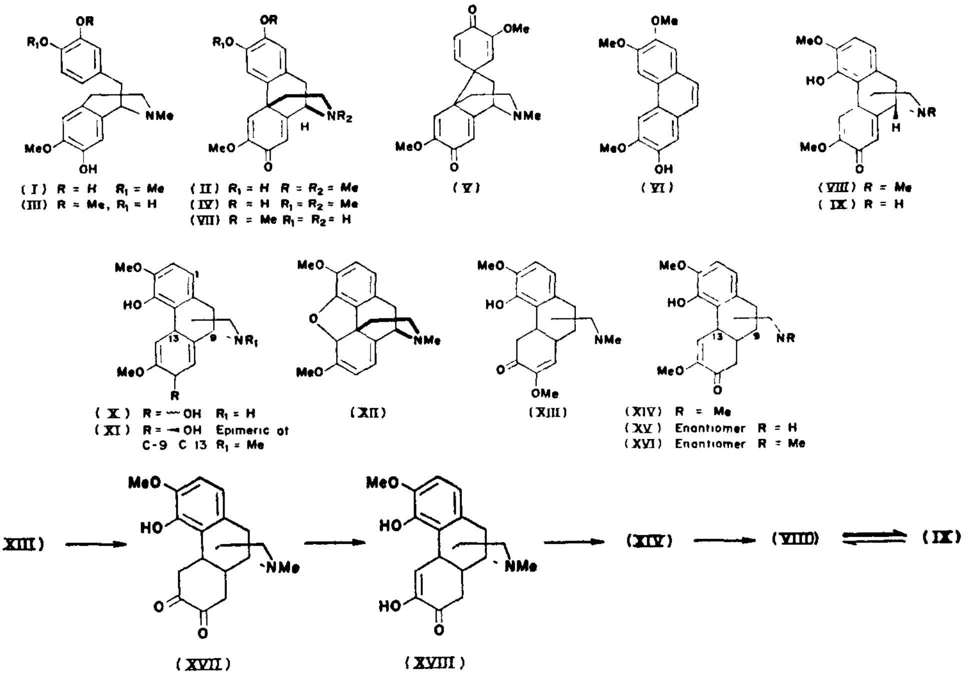
dienones. In all these experiments, the location of tritium in the isolated norsinoacutine was shown to be at C-1 by the fact that bromination at this site removed all the radioactivity. In the case of sinoacutine, the location of the label was ascertained by deuteration, and more than 97% of the activity was removed in this derivative.

TABLE 1. ACTIVITY OF COMPOUNDS FED TO *Croton flavens* AND PERCENTAGE INCORPORATION

Compound fed	Activity (μCi)	Alkaloid extracted	% incorporation
1-^{3}H-Sinoacutine	52.2	Norsinoacutine	0.20
1-^{3}H-Norsinoacutine	45.1	Sinoacutine	0.01
1-^{3}H-Norsinoacutinols	50.8	Norsinoacutine	0.002
		Sinoacutine	0.001
L-[*S*-Methyl-^{14}C]methionine	50.0	Sinoacutine	0.0002
		Norsinoacutine	0.001*
		Flavinantine	0.001†
Glycine-2-^{14}C	1.0	Norsinoacutine	0.0002
		Flavinantine	0.0003
Glycine-2-^{14}C	1.0	Norsinoacutine	0.001
		Flavinantine	0.003‡
1-^{3}H-Sinomenine	57.0	Norsinoacutine	0.002
1-^{3}H-Sinomenine	63.0	Sinoacutine	0.001
1-^{3}H-Sinomenine	33.0	Norsinoacutine	0.001
1-^{3}H-Isosinomenine	14.0	Norsinoacutine	0.01
(±)-[2-^{14}C]-Phenylalanine	20.0	Flavinantine	0.001
		Sinoacutine	0.001
		Norsinoacutine	0.002
(±)-[2-^{14}C]-Phenylalanine	30.0	Flavinantine	0.001
		Sinoacutine	0.001
		Norsinoacutine	0.005
(±)-[*N*-Methyl-^{14}C]orientaline	9.0	Flavinantine	0.012
		Sinoacutine	0.0002
		Norsinoacutine	Inactive
(±)-[*N*-Methyl-^{14}C]reticuline	10.4	Flavinantine	0.103
		Sinoacutine	0.002
		Norsinoacutine	Inactive
1-^{3}H-8,14-Dihydronorsalutaridine	58.0	Norsinoacutine	Inactive
		Flavinantine	Inactive
1-^{3}H-8,14-Dihydrosalutaridine	58.0	Norsinoacutine	Inactive
		Sinoacutine	Inactive

* Activity in 2 × O-Me (%) 92, N-Me (%) Groups 0. Total (%) 92.
† Activity in 2 × O-Me (%) 60, N-Me (%) Groups 29, Total (%) 89.
‡ Activity in 2 × O-Me (%) 60.2, N-Me (%) Groups 25, Total (%) 85.2.

Because of the demonstrated role of sinoacutine in the formation of sinomenine (XIII),[5] it was decided to investigate whether or not *C. flavens* could utilize 1-^{3}H-sinomenine for the elaboration of norsinoacutine and sinoacutine. Table 1 indicates that these transformations are possible. In addition it was shown that 1-^{3}H-isosinomenine (XIV) was a better precursor and Scheme 1 outlines how these compounds may be converted to norsinoacutine. It should, however, be borne in mind that the presence of sinomenine and isosinomenine in *C. flavens* has not yet been demonstrated. In cases where the presence of a precursor has not been established in a plant, the experiment really only indicates that the plant system being studied is capable of accomplishing a particular transformation, and the compound may not necessarily be an intermediate along the main biosynthetic pathway to the isolated radioactive compound.



SCHEME 1 SUGGESTED TRANSFORMATION OF SINOMENINE TO NORSINOACUTINE

In order to investigate the “C_1” pool of this plant, the facility of the plant to utilize methionine and glycine as precursors of the *O*- and *N*-methyl groups was checked These compounds were fed as L-[S-methyl-^{14}C]methionine and glycine-2-^{14}C, and both were incorporated into the alkaloids under study Sinoacutine was not recovered from the glycine feedings and it is now known that the production of sinoacutine varies with the season[6] and no sinoacutine was isolated when this experiment was performed Norsinoacutine and flavinantine recovered from the methionine experiment were demethylated using the method described recently by Jeffs *et al*[7] In both cases, the activity was shown to be located primarily in the methyl groups (92 and 89·5 % respectively) In the glycine feedings only the alkaloid flavinantine isolated from the second experiment was demethylated, and this showed that 85 2 % of the total activity was located in the methyl groups The results of these experiments show that both methionine and glycine can serve as precursors of labile methyl groups in *C flavens*, and the results also suggest that as a source of one carbon unit, these precursors are at equal levels along the biosynthetic pathway

Both 1-^{3}H-8,14-dihydronorsalutaridine (XV) and 1-^{3}H-8,14-dihydrosalutaridine (XVI) were fed and norsinoacutine, flavinantine in the case of the former compound and norsinoacutine and sinoacutine in the latter case were isolated and shown to be radioisotopically

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[6] STUART, K L and GRAHAM, L , unpublished results.

[7] JEFFS, P W , ARCHIE, W C , HAWKS, L and FARRIER, D S (1971) *J Am Chem Soc* **93**, 3752

inactive. This finding is not surprising since in the case of flavinantine a radical modification of the substitution pattern of the aromatic ring would be required, and for norsinoacutine and sinoacutine to be formed from these compounds, the plant would have to accomplish not only the oxidation step, but in addition, the more difficult inversion of two asymmetric centres, one at C-9 and the other at C-13.

From these feeding experiments it now seems very likely that in *C. flavens*, the major pathway to the alkaloid flavinantine is from the amino acids phenylalanine and glycine or methionine and then via reticuline and isosalutaridine. Although the interconvertibility of sinoacutine and norsinoacutine have been demonstrated, the greater predominance of norsinoacutine clearly indicates a biosynthetic bias in favour of this alkaloid.

EXPERIMENTAL

Croton flavens L. plants were grown in the Botany gardens, U.W.I., Mona, Jamaica in the open. A special water-proof cellophane sack was developed and used to protect the wick and feeding vial from rain and insects. All radioisotopic feedings were by the wick-insertion under the bark method and the feed limb was isolated usually 2 weeks after feeding. It was earlier shown by feeding radioactive phenylalanine that there was no redistribution of any radioactivity to any other limbs in the fed plant during a 2-week period. In some cases, the sparing solubility of the test compound necessitated the use of a drop of 3 N HCl and a drop of EtOH for a 10 mg sample. During isolation, when necessary, TLC and PLC were done using silica as adsorbent and column chromatography was carried out on grade II–III neutral alumina. All counts (radioactivity) were carried out on a Tri-Carb liquid scintillation counter, model 314 EX-2 (Packard), or a Beckman liquid scintillation counter, Model LS 150. Samples were prepared for counting by dissolving the crystals in a minimum of MeOH and adding this to the scintillation solution (10 ml) which consisted of POP and POPOP in toluene. All samples were crystallized to constant activity and incorporation percentages calculated on duplicate counts.

Preparation of 1-^{3}H-*sinoacutine and feeding*. A solution of sinoacutine (100 mg) in dimethylformamide (2 ml) and 1 ml tritiated H_2O of activity 0.2 Ci were sealed under nitrogen in a thick glass tube. The tube was heated at 100° (72 hr) in a pressure tube, then cooled to room temp., chilled in ice and opened. After dilution with H_2O, the contents of the tube were extracted with $CHCl_3$ and the brown oil resulting put on an alumina column and eluted with $CHCl_3$. Crystallization from EtOH yielded 1-^{3}H-sinoacutine (13.8 mg) having an activity of 5.55 μCi/mg. 9.4 mg of this material was dissolved in a mixture consisting of 1 drop EtOH, 1 drop 3 N HCl and H_2O, and fed to the plant.

Isolation of alkaloids after feeding radioactive material. In a typical experiment, the reaped branch was dried and powdered (230 g) and digested with 2% tartaric acid solution (2 l.). The acid extract was concentrated at reduced pressure, placed in an ice-box overnight, filtered, basified with 25% NH_4OH and then continuously extracted with $CHCl_3$ (2 days). After evaporation, 2.64 g of crude alkaloid was obtained. This was dissolved in $CHCl_3$ and a few drops of MeOH, put on an alumina column (14 × 4.5 cm dia.) packed in $CHCl_3$. Elution with $CHCl_3$ yielded sinoacutine (0.058 g). $CHCl_3$–MeOH (95:5) first removed a brown band which was followed by a yellow band. These when combined (based on TLC evidence) yielded norsinoacutine (0.49 g). Further elution (150 ml) gave a fraction containing flavinantine (0.22 g). All alkaloids isolated were compared with authentic samples.

Bromination of norsinoacutine. ^{3}H-Norsinoacutine (28.1 mg) as isolated from an appropriate feeding experiment was diluted with unlabelled material and the whole (233 mg) dissolved in $CHCl_3$ and Br_2 (187 mg) in $CHCl_3$ (10 ml) was added dropwise with stirring over 25 min. Stirring was continued for a further 5 hr. The $CHCl_3$ solution was washed with 1 N $NaHCO_3$ and then H_2O. After evaporation the dark residue was passed through an alumina column, eluted with $CHCl_3$–MeOH (1:1). The product had an IR spectrum identical to that of an authentic sample. Counting of the bromo compound (5 mg) showed that all the radioactivity was removed, and proving that the tritium label was exclusively at C-1.

Incorporation of 1-^{3}H-*norsinoacutine*. Radioactive norsinoacutine was prepared in a similar manner to that described for sinoacutine. 9.5 mg (4.75 μCi/mg) was fed and sinoacutine isolated and recrystallized by dissolving the sample in EtOAc–$CHCl_3$ (5:1) with warming and then causing rapid cooling by bubbling N_2 through the solution. An incorporating of 0.01% was obtained.

Experiment with 1-^{3}H-*norsinoacutinols*. Norsinoacutine (216 mg) was tritiated as outlined for sinoacutine and 100 mg of the product as dissolved in EtOH (10 ml). $NaBH_4$ (340 mg) was added to the solution which was maintained at 0° for 4 hr. After a further 2 hr at room temp. 2 N HCl was added dropwise until effervescence ceased. The solution was then adjusted to pH 9 with NH_4OH and the mixture extracted with $CHCl_3$. Evaporation yielded two epimeric alcohols (TLC). 12 mg of this mixture of alcohols (4.23 μCi/mg) was fed to the plant and reaped 2 weeks later. The extraction of the crude alkaloid mixture and then the separation of sinoacutine and norsinoacutine were carried out as described above (see Table 1 for % incorporation)

*Demethylation of alkaloids from glycine-*2-^{14}C *and* L-[S-*methyl*-^{14}C] *methionine* The demethylation of flavinantine derived from the methionine feeding experiment will be described as a typical example The Pregl–Lieb apparatus was modified for sequential *O*- and *N*-methyl microdeterminations,[7] so that the reaction flask (B) preceding the washer-receiver train was extended through a Claisen head adapter to another reaction flask (A) The washer was charged with NaOAc (25%) and $CdSO_4$ (5%) (10 1) The receiver contained ethanolic NMe_3 (10%) and was cooled in solid CO_2–acetone bath Flavinantine (30 mg) was dissolved in Ac_2O (0 1 ml) a crystal of phenol, a small square of aluminium foil and freshly distilled HI (2 ml, sp gr 1 7) were added to flask (A) A stream of N_2 passed through pyrogallol, then conc H_2SO_4 was adjusted to a flow rate of 1 bubble/sec The solution was refluxed (1 hr) and then all but 0 5 ml allowed to distil into flask (B) over 10 min This completed the *O*-demethylation process After removing the receiver, it was warmed to room temp and evaporated under reduced pressure This afforded NMe_4I (25 8 mg) A new receiver was attached and NH_4I (10 mg) was added to the cooled residue in flask A Three drops of catalytic chloroauric acid (3 4%) were introduced, and the remainder of the residue was distilled into flask B and the residual salt pyrolysed (90 min) The solution in the receiver on being warmed to room temp under reduced pressure afforded NMe_4I (2 95 mg), derived from the *N*-methyl group Scintillation counting showed that 60% of the activity was in the *O*-methyl groups and 29% in the *N*-methyl group

Tritiation of sinomenine and preparation of 1-^{3}H-*isosinomenine* Sinomenine (500 mg) was dissolved in $HCONMe_2$ (2 5 ml), and 0 5 ml tritiated H_2O of activity 0 1 Ci along with sodium formate (103 mg) were added[5] The mixture was sealed under N_2 and heated at 100° for 7 days The solution was then evaporated and the residue treated with potassium-*t*-butoxide (700 mg) in H_2O (10 ml) under N_2 overnight at room temp An excess of solid CO_2 was added to the solution and the liberated sinomenine was extracted into $CHCl_3$ Sinomenine (277 mg) was separated from other products on a PLC plate Some of this product (100 mg) was dissolved in MeOH (3 ml) and $CHCl_3$, freshly distilled from P_2O_5 (96 ml) and previously saturated with dry HCl, added The resulting solution was kept overnight at 10°, then agitated under reduced pressure to remove HCl, and finally shaken with an excess of 1 N $NaHCO_3$ The aqueous layer was extracted with $CHCl_3$, and the dried solution was evaporated The residue was separated on very thin TLC plates in $CHCl_3$–MeOH (3 1) The faster running band was extracted and after recrystallization yielded pure isosinomenine This was incorporated to the extent of 0 01% into norsinoacutine

Deuteration of sinoacutine A solution of radioactive sinoacutine (25 mg) in $HCONMe_2$ and D_2O (2 ml) was sealed with N_2 in a glass tube The tube was placed in a stainless steel bomb and heated at 100° for 72 hr The tube was cooled to room temp, chilled in ice and opened The solution was diluted with water and extracted with $CHCl_3$ $CHCl_3$ was evaporated and traces of $HCONMe_2$ removed at 50° under reduced pressure Purification on a small alumina column and subsequent crystallization from EtOAc gave 1-^{2}H-sinoacutine Scintillation counting showed that less than 3% of the activity remained

Synthesis of (+)-[N-*methyl*-^{14}C]*reticuline* The procedure used was essentially that as reported earlier[8] The purity of the labelled reticuline was ascertained after TLC on a radioisotope scanner 115 mg of the pure product obtained has an activity of 1 14 × 10^{-1} μCi/mg

Synthesis of (±)-[N-*methyl*-^{14}C]*orientaline* HCl This synthesis was similar to that carried out by Battersby *et al*[9] and yielded 160 mg of material (1 13 × 10^{-1} μCi/mg) A radioisotope scan of TLC showed only one radioactive component to be present

Degradation of flavinantine from (±)-[2-^{14}C]*phenylalanine feeding* Flavinantine (90 mg) from both feeding experiments using radioactive phenylalanine was combined and recrystallized to constant activity (5 04 × 10^{-3} μCi/mmol) This material was converted to NO-dimethylflavinantine,[2] and the quaternary base (57 mg) treated with 20% NaOH (5 ml) and heated at 120–140° (1 hr) in N_2 The alkaline solution was extracted with $CHCl_3$ to yield 8 7 mg of a phenol, which recrystallized from MeOH to yield cream-coloured crystals, m p 186–190° Dimethylaminoethanol produced by this degradation was trapped as the chloroaurate An alternative method was used in view of the low yield of the desired phenolic compound NO-dimethylflavinantine sulphate was first prepared by dissolving radioactive flavinantine (80 mg) in MeOH, adding Me_2SO_4 (0 5 ml) followed by NaOH solution until the mixture was just basic The mixture was allowed to stand overnight This was subject to Hofmann degradation without separation The solution on cooling was extracted with $CHCl_3$ and after acidification was again extracted with $CHCl_3$ Both fractions were shown to contain an identical major product by TLC and were combined and purified on neutral alumina in $CHCl_3$ Crystals obtained (25 mg) had m p 186–188°, λ_{max}^{EtOH} 218, 255, 286, 300, 335, 350 nm (log ϵ 4 36, 4 86, 4 38, 4 16, 3 08, 2 78) UV data is characteristic of a phenanthrene (Found C, 69 8, H, 6 1 $C_{17}H_{16}O_4\ \frac{1}{2}H_2O$ requires C, 69 7, H, 5 8%) The activity of this phenanthrene was shown to be 2 36 × 10^{-4} μCi/mmol and

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[8] Barton, D H R, Kirby, G W, Steglich, W, Thomas, G M, Battersby, A R, Dobson, T A and Ramuz, H (1965) *J Chem Soc*. 2423

[9] Battersby, A R, Brown, T H and Clements, J H. (1965) *J Chem Soc* 4550

so accounted for 47% of the activity of flavinantine Due to the very dark colour of the chloroaurate of dimethylaminoethanol, no reliable counts could be obtained on this material, especially in view of its low activity

Degradation of flavinantine from feeding experiments with $(\pm)$-[N-*methyl*-^{14}C]*orientaline* Flavinantine (96 mg, $1\ 50 \times 10^{3}$ dpm/mmol) isolated from this feeding experiment was degraded as described above to yield 2-hydroxy-3,6,7-trimethoxyphenanthrene (20 mg) which was identical (m p , TLC) to that obtained earlier This material contained no activity

Degradation of flavinantine from feeding experiment using $(\pm)$-[N-*methyl*-^{14}C]*reticuline* Flavinantine (85 mg), $2\ 27 \times 10^{4}$ dpm/mmol was also degraded to the phenanthrene described above, but it showed no activity and attested to a lack of randomization of the *N*-methyl label during the duration of the experiment

Tritiation of 8,14-*dihydronorsalutaridine and* 8,14-*dihydrosalutaridine* 8,14-Dihydronorsalutaridine (85 mg) was tritiated in the usual way to yield 25 mg (9 7 μCi/mg) of 1-^{3}H-8,14-dihydronorsalutaridine 8,14-Dihydrosalutaridine with an activity of 58 μCi/mg was also prepared and both fed to *C flavens* In both cases, alkaloids isolated were shown to be inactive

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