Phenol Oxidation and Biosynthesis. Part XIV.* (Alkaloids from Croton Species. Part VII.†) The Biosynthesis of Crotonosine [1]

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The incorporation of (±)-, (+)-, and (−)-coclaurine into crotonosine in *Croton linearis* Jacq. has been investigated and the specific utilisation of the (+)-isomer demonstrated. The isomeric (±)-isococlaurine is not a precursor of crotonosine although (±)-norcoclaurine is incorporated. The evidence supports the oxidative cyclisation of (+)-coclaurine to a dienone. Double labelling experiments involving the methoxy-group of (±)-coclaurine showed that most, but not all, of the methoxy-activity was lost in conversion into crotonosine; the significance of these results is discussed.

ACCORDING to biogenetic theory [2] the proaporphine alkaloid crotonosine (I) [3] should be formed in Nature by oxidative cyclisation of coclaurine (II; $R^1 = Me$, $R^2 = H$). We now report experimental confirmation of this idea.

(±)-Coclaurine was labelled with tritium, as shown in (II; $R^1 = Me$, $R^2 = H$), by base-catalysed exchange in tritiated water at 100°.[4] Heating was prolonged to

* Part XII, *J. Chem. Soc.* (*C*), 1967, 128. The Paper by D. H. R. Barton, R. James, G. W. Kirby, W. Döpke, and H. Flentje, *Chem. Ber.*, in the press, is regarded as Part XIII of this Series.

† Part VI, *J. Chem. Soc.* (*C*), 1967, 154.

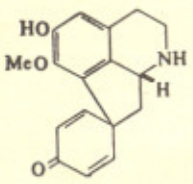
[1] Preliminary communications, L. J. Haynes, K. L. Stuart, D. H. R. Barton, D. S. Bhakuni, and G. W. Kirby, *Chem. Comm.*, 1965, 141; D. H. R. Barton, *Pure Appl. Chem.*, 1964, **9**, 35.

[2] D. H. R. Barton and T. Cohen, "Festschrift A. Stoll," Birkhauser, Basel, 1957, p. 117.

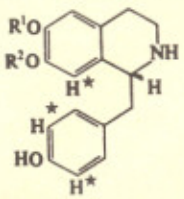
[3] L. J. Haynes, K. L. Stuart, D. H. R. Barton, and G. W. Kirby, *J. Chem. Soc.* (*C*), 1966, 1676.

[4] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.

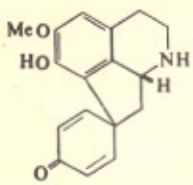
ensure essentially equal labelling at the three exchangeable positions. Degradation of a diluted sample confirmed the equality of labelling; methylation and



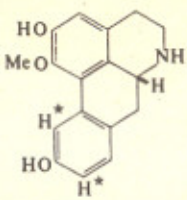
(I)



(II)



(III)



(IV)

Hofmann elimination gave the corresponding stilbene which was oxidised to anisic acid containing 66% of the total ^3H activity. (±)-[$^3H_3$]Coclaurine was fed to mature *Croton linearis* Jacq. plants (growing in the West Indies) and reproducible incorporation into crotonosine was observed. The results of several feedings are recorded in Table 1; incorporation values are corrected for the obligatory loss of one tritium. (±)-Nor[$^3H_4$]-coclaurine, obtained by base catalysed tritiation of (±)-norcoclaurine (II; $R^1 = R^2 = H$), was also incorporated, though less efficiently, into crotonosine. In contrast, no incorporation of (±)-[$^3H_3$]isococlaurine, obtained by base-catalysed tritiation of (±)-isococlaurine (II; $R^1 = H$, $R^2 = Me$), was observed. This negative result supports the original theory [2] which demands cyclisation *ortho* and *para* to free phenolic hydroxy-groups. Labelled crotonosine, derived from (±)-[$^3H_3$]coclaurine, was converted into *NO*-diacetylcrotonosine which had the same molar activity. Reduction to the tetrahydro-derivative, treatment with hot methanolic sodium hydroxide, and reacetylation gave inactive *NO*-diacetyltetrahydrocrotonosine. Thus all the tritium was, as expected, α to the carbonyl group in crotonosine. In a similar feeding experiment (±)-[2-^{14}C]phenylalanine was also incorporated (0·04%) into crotonosine.

Resolution of (±)-*OO'*-dibenzylcoclaurine was effected with both (+)- and (−)-dibenzoyltartaric acid. Hydrogenolysis then gave the corresponding (see Table 2) optically pure forms of coclaurine. The chirality of each form was established by conversion into the corresponding *N*-methyl derivative of known [5-7] absolute configuration. In this way it was shown that (+)-coclaurine has the absolute configuration as in (II). Feeding experiments with (+)- and (−)-coclaurine, labelled with tritium in the usual way, showed (Table 1) that only (+)-coclaurine was an efficient precursor of crotonosine. This confirms the absolute configuration of the alkaloid and shows that racemisation [8] of coclaurine is unimportant in *Croton linearis*.

Oxidation of coclaurine (II; $R^1 = Me$, $R^2 = H$) would give the dienone (III) isomeric with crotonosine (I). If coclaurine is indeed the immediate precursor of the proaporphine ring system then we must explain the transformation (III) ⟶ (I). Two possibilities can be considered: first, demethylation to give a catechol followed by remethylation of the alternative hydroxy-group, and secondly, migration of a methyl group (possibly by formation and cleavage of a methylene function) from one oxygen to the next. To distinguish between these possibilities (±)-[*methyl*-^{14}C,$^3H_3$]coclaurine was fed to *Croton linearis*. The $^3H/^{14}C$ ratio in the precursor was set at 1·8. According to the methyl migration hypothesis, the biosynthetically derived crotonosine should have had an activity ratio of 1·2, since one third of the tritium is lost during cyclisation. The experimental value was 4·0. This corresponds to a loss of 70% of the methoxy-^{14}C activity during biosynthesis. Zeisel demethylation gave methyltriethylammonium iodide containing 89% of the ^{14}C activity present in the crotonosine. Demethylation of the derived apocroto-

TABLE 1

Incorporation of coclaurine derivatives into crotonosine

Precursor	(±)-Coclaurine	(+)-Coclaurine	(−)-Coclaurine	(±)-Norcoclaurine	(±)-Isococlaurine
Incorporation (%)	0·19, 0·20, 0·11 *	0·17 †	0·00,† 0·00 †	0·08, 0·11, 0·07	0·00 *

Incorporation corrected for loss of one tritium; * and † indicate feeding experiments performed in parallel.

TABLE 2

$[\alpha]_D$ Values for coclaurine derivatives (in methanol)

	(+)-Coclaurine series	(−)-Coclaurine series
OO'-Dibenzylcoclaurine (+)-dibenzoyltartrate		+72°
OO'-Dibenzylcoclaurine (−)-dibenzoyltartrate	−72°	
OO'-Dibenzylcoclaurine	−16, +24·5 *	+15, −26·5 *
OO'-Dibenzylcoclaurine hydrochloride	−48	+47
Coclaurine hydrochloride	+13	−14
N-Methylcoclaurine ...	−120 (lit.,[6] −122)	+123 (lit.,[6] +124)
NOO'-Trimethylcoclaurine	−83 (lit.,[7] −85·7)	+83 (lit.,[7] +86·3)

* In chloroform.

[5] C. Ferrari and V. Deulofeu, *Tetrahedron*, 1962, **18**, 419.
[6] H. Yamaguchi, *J. Pharm. Soc. Japan*, 1958, **78**, 678.
[7] M. Tomita and J.-I. Kunitomo, *J. Pharm. Soc. Japan*, 1962, **82**, 734.
[8] Cf. A. R. Battersby, D. M. Foulkes, and R. Binks, *J. Chem. Soc.*, 1965, 3323.

nosine (IV) gave a value of 92% for the methoxy-^{14}C content. This feeding experiment was repeated using doubly labelled coclaurine with a $^{3}H/^{14}C$ ratio of 13·0. The derived crotonosine was counted accurately for tritium (the dominant isotope) and demethylated to give methyltriethylammonium iodide which was counted for ^{14}C. The observed $^{3}H/^{14}C$ ratio was 21·5, corresponding to a 60% loss of methoxy-activity during biosynthesis. Conversion into apocrotonosine and base-catalysed exchange caused loss of essentially all (97%) of the tritium, in agreement with the expected labelling pattern (IV). The partial and variable incorporation of the coclaurine methoxy-group into crotonosine is explicable by the demethylation–remethylation hypothesis bearing in mind the " anomalous " results recently obtained by Austin and Meyers [9] with a doubly labelled precursor of the coumarin skimmin. Thus the results we have observed might depend on the size of the " methyl " pool at the site of biosynthetic activity for the conversion of (III) into (I). Very much more detailed experimentation would be needed to establish this point unambiguously. However, in any case, *Croton linearis* Jacq. is not a suitable plant for such detailed investigation.

EXPERIMENTAL

Melting points were determined with a Kofler hot-stage apparatus. Nuclear magnetic resonance spectra were run on a Varian A-60 spectrometer on permanent loan to one of us (D. H. R. B.) from the Wellcome Trust.

Counting Methods.—Liquid scintillation counting was used for the measurement of ^{3}H and ^{14}C, an Isotope Development Ltd. (type 6012A) or a Packard (model 314 EX) instrument being employed.

Synthesis of Benzylisoquinoline Precursors.—The racemates of coclaurine,[10] norcoclaurine,[11] and isococlaurine [12] were prepared by standard methods. Modified procedures and new intermediates are recorded below.

OO′-Dibenzylcoclaurine.—Prepared by reduction of the corresponding dihydroisoquinoline with sodium borohydride in methanol, *OO′*-dibenzylcoclaurine crystallised from ethanol as plates, m. p. 87° (lit.,[13] 82—83°) (Found: C, 79·6; H, 6·7; N, 3·05. Calc. for $C_{31}H_{31}NO_3$: C, 80·0; H, 6·7; N, 3·0%).

N-(3,4-Dibenzyloxyphenethyl)-4-benzyloxyphenylacetamide.—4-Benzyloxy-ω-diazoacetophenone [14] (1 g.) and 3,4-dibenzyloxyphenethylamine (1·7 g.) [15] in dry benzene (150 ml.) were irradiated with u.v. light in the usual way.[16] Chromatography of the product on alumina (grade III) gave the desired *amide* as needles (1·1 g.), m. p. 125° (from methanol) (Found: C, 79·9; H, 6·3; N, 2·7. $C_{37}H_{35}NO_4$ requires C, 79·7; H, 6·3; N, 2·5%).

OO′O″-Tribenzylnorcoclaurine.— The aforementioned amide was cyclised with phosphoryl chloride, in the usual way, to give the corresponding 3,4-dihydroisoquinoline. Reduction with sodium borohydride in methanol then gave *OO′O″-tribenzylnorcoclaurine* as an oil. Chromatography on alumina (grade III) and crystallisation from methanol gave plates, m. p. 89° (Found: C, 82·2; H, 6·6; N, 2·6. $C_{37}H_{35}NO_3$ requires C, 82·1; H, 6·5; N, 2·6%).

Resolution of OO′-Dibenzylcoclaurine.—The salt from (±)-*OO′*-dibenzylcoclaurine (1·74 g.) and (+)-dibenzoyltartaric acid (1·34 g.) was fractionally crystallised from, successively, benzene–ether, benzene, ethanol–ether, ethanol, and methanol to give needles (0·95 g.), $[\alpha]_D$ +72° (*c* 0·94 in methanol). The salt appeared to be polymorphic; m. p.s of 113, 142—143, and 162—165° were obtained with different samples. The rotation, however, was constant. The salt was decomposed with sodium hydroxide and the liberated *OO′*-dibenzylcoclaurine chromatographed on alumina (grade III) and crystallised from ethanol to give plates, m. p. 86—87°, $[\alpha]_D$ +15° (*c* 0·5 in methanol) and −26·5° (*c* 0·8 in chloroform). The corresponding hydrochloride crystallised from ethanol as needles (dried *in vacuo* over P_2O_5), m. p. 170—171°, $[\alpha]_D$ +47° (*c* 0·1 in methanol).

(−)-Coclaurine Hydrochloride.— (+)-*OO′*-Dibenzylcoclaurine hydrochloride (230 mg.) was hydrogenated in ethanol (2 ml.) using 10% palladium–carbon catalyst (115 mg.). The resulting (−)-coclaurine hydrochloride crystallised from ethanol as needles, m. p. 165—166°. Dried at 100° *in vacuo* for 25 hr. this hydrochloride had m. p. 247—248°, $[\alpha]_D$ −14° (*c* 1·1 in methanol). The free base had $[\alpha]_D$ −16° (*c* 1·0 in methanol).

(+)-N-Methylcoclaurine.—(−)-Coclaurine hydrochloride (100 mg.) in water (1 ml.) was treated under nitrogen successively with 2N-sodium hydroxide (1 ml.), formic acid (1·2 ml.; 98%), and aqueous formaldehyde (1·2 ml.; 37—41%), at pH *ca.* 5. The mixture was heated at 100° for 15 min. and the basic products isolated in the usual way. Chromatography on alumina (grade III; 25 g.) and elution with chloroform–ethanol (94 : 6) gave (+)-*N*-methylcoclaurine (60 mg.), m. p. 178°, $[\alpha]_D$ +123° (*c* 0·5 in methanol) (lit.,[6] m. p. 178—179°, $[\alpha]_D$ +124°).

(+)-NOO′-Trimethylcoclaurine.—(+)-*N*-Methylcoclaurine (50 mg.) in methanol (0·3 ml.) was treated with an excess of ethereal diazomethane for 3 days. Chromatography of the basic products on alumina (grade III; 10 g.) and elution with benzene–chloroform (3 : 2) gave (+)-*NOO′*-trimethylcoclaurine (25 mg.), m. p. 60°, $[\alpha]_D$ +83° (*c* 0·3 in ethanol) (lit.,[7] m. p. 61—62°, $[\alpha]_D$ +86°).

(+)-Coclaurine Hydrochloride.—*OO′*-Dibenzylcoclaurine, enriched with the (+)-enantiomer (from the resolution described above), was converted into the corresponding (−)-dibenzoyltartrate. Thereafter, preparation of compounds in the (+)-coclaurine series proceeded as before. Rotations are given in Table 2; m. p.s were close to those of the corresponding enantiomers.

Labelling of Precursors.—Tritiation. (±)-Coclaurine hydrochloride (110 mg.) in tritiated water (0·5 ml.) containing potassium t-butoxide (150 mg.) was heated under nitrogen (sealed tube) for 80 hr. at 100°. The product was diluted with water and treated with an excess of carbon dioxide to precipitate [3′,5′,8-$^{3}H_3$]coclaurine, which was purified as the hydrochloride (55 mg.). Dilution with inactive hydrochloride and repeated crystallisation from

[9] D. J. Austin and M. B. Meyers, *Chem. Comm.*, 1966, 125.
[10] K. Kratzl and G. Billek, *Monatsh.*, 1951, **82**, 568; M. Tomita, K. Nakaguchi, and S. Takagi, *J. Pharm. Soc. Japan*, 1951, **71**, 1046.
[11] H. Yamaguchi, *J. Pharm. Soc. Japan*, 1958, **78**, 692.
[12] M. Tomita and H. Yamaguchi, *J. Pharm. Soc. Japan*, 1952, **72**, 1219.
[13] H. Yamaguchi and K. Nakano, *J. Pharm. Soc. Japan*, 1959, **79**, 1106.
[14] J. A. D. Jeffreys, *J. Chem. Soc.*, 1956, 4451.
[15] E. J. Forbes, *J. Chem. Soc.*, 1955, 3926.
[16] Cf. D. H. R. Barton, G. W. Kirby, W. Steglich, G. M. Thomas, A. R. Battersby, T. A. Dobson, and H. Ramuz, *J. Chem. Soc.*, 1965, 2423.

methanol–ether did not cause loss of activity. Deuteriation under the same conditions showed (n.m.r. control) exchange of 3 aromatic protons. The other benzylisoquinoline precursors were tritiated in the same way.

With ^{14}C. 4-Benzyloxy-[*methyl*-^{14}C]-3-methoxy-benzaldehyde was used to prepare (±)-[*methyl*-^{14}C]coclaurine by standard procedures.[16]

Degradation of (+)-[3′,5′,8-$^{3}H_3$]*Coclaurine*.—Tritiated (±)-coclaurine hydrochloride (200 mg.) [relative molar activity (r.m.a.) 1·00] in dimethylformamide (7 ml.) was treated under nitrogen with sodium hydride (100 mg.) and methyl iodide (2 ml.). After 18 hr., the solvent was evaporated under reduced pressure and the product was extracted into chloroform. Evaporation gave *OO*′-dimethylcoclaurine methiodide, as needles, m. p. 112—114° (from ethanol–ether) (lit.,[17] 110—112°). The methiodide was heated under reflux in aqueous potassium hydroxide (20%; 7 ml.) for 20 hr. The product was extracted into ether (6 × 5 ml.) and chromatographed on alumina (grade III; 25 g.). Elution with benzene–chloroform (5 : 1) gave the corresponding stilbene as needles, m. p. 82—85° [from ether–light petroleum (b. p. 60—80°)] [lit.,[17] m. p. 82—85° (r.m.a. 1·01)]. The stilbene (50 mg.) was ozonised at 0° in methylene chloride (8 ml.). The solvent was evaporated and the ozonide, in ether (60 mg.), was treated, with stirring, with a suspension of silver oxide (100 mg.) in aqueous sodium hydroxide (10%; 10 ml.) for 30 min. at room temperature, then at 100° for 2 hr. The solution was filtered and the filtrate acidified. Extraction with ether gave anisic acid (11 mg.) (r.m.a. 0·66).

Feeding Experiments.—Precursors were introduced in aqueous solution, pH *ca.* 6, by means of cotton wicks passed into the limbs of *Croton linearis* plants. Well established plants growing naturally in the West Indies were selected for this purpose. After a suitable period (2—3 weeks) for metabolism the limbs were cut off and extracted for crotonosine.

Isolation and Purification of Crotonosine.—A limb (typically 100 g. wet wt.) of *Croton linearis* was macerated with 2% aqueous tartaric acid (2 l.) for 4 hr. The mixture was filtered and the filtrate concentrated to *ca.* 250 ml. The solution was basified (pH 8) with ammonium hydroxide and extracted continuously (24 hr.) with chloroform. Concentration and seeding of the extract gave crotonosine (typically 100 mg.). The alkaloid was purified by crystallisation from acetone–methanol (3 : 1) and further by conversion into the diacetyl derivative and hydrochloride.

Degradation of Crotonosine.—Tritiated crotonosine derived from (±)-[3′,5′,8-$^{3}H_3$]coclaurine was converted into diacetyltetrahydrocrotonosine.[18] Treatment with refluxing 5% methanolic sodium hydroxide for 24 hr. followed by reacetylation gave inactive material. Radioactive crotonosine from the doubly labelled coclaurine feeding (see previously) was converted into apocrotonosine in the usual way.[3] Base-catalysed exchange (see labelling of benzylisoquinolines) removed essentially all (97%) of the tritium. The methoxy-^{14}C activity of crotonosine or apocrotonosine was determined by Zeisel demethylation; methyl iodide was trapped and counted as methyltriethylammonium iodide.

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[17] M. Tomita, Y. Inubushi, and M. Yamagara, *J. Pharm. Soc. Japan*, 1951, **71**, 1069.
[18] L. J. Haynes and K. L. Stuart, *J. Chem. Soc.*, 1963, 1789.