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BIOSYNTHESIS OF CROTSPARINE, CROTSPARININE AND SPARSIFLORINE*

DEWAN S. BHAKUNI, SHEO SATISH, HEMA UPRETY and RANDHIR S. KAPIL

Central Drug Research Institute, Lucknow, India

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Key Word Index—*Croton sparsiflorus*, Euphorbiaceae, crotsparine, crotsparinine, sparsiflorine, alkaloid biosynthesis

Abstract—The biosynthesis of crotsparine, crotsparinine and sparsiflorine in *Croton sparsiflorus* has been studied using racemic [Ar-^{3}H]-coclaurine, isococlaurine and norcoclaurine Tyrosine and coclaurine are shown to be precursors of all three alkaloids

RECENTLY we reported[1] the isolation of the proaporphine alkaloids crotsparine (**1**), *N*-methylcrotsparine (**2**), and *N*,*O*-dimethylcrotsparine; dihydroproaporphines crotsparinine (**3**) and *N*-methylcrotsparinine (**4**) and the aporphine sparsiflorine[2] (**5**) from *Croton sparsiflorus*, a South American weed which has now completely naturalized throughout the plains of India. Of the isolated bases *N*-methylcrotsparine is a potent hypotensive agent[3]; *N*-methylcrotsparinine produces an initial sharp transient fall of blood pressure followed by a gradual sustained hypotension at 3–5 mg/kg dose; and *N*-methylsparsiflorine methiodide also produces hypotension at 5–10 mg/kg. The magnitude of hypotension in this case was similar to that of *N*-methylcrotsparine. The structure and stereochemistry of proaporphine and dihydroproaporphine bases from *C. sparsiflorus* have been determined.[1] The absolute configuration of these bases has been established[4] by chemical correlation. The present communication describes the biosynthesis of crotsparine, crotsparinine and sparsiflorine.

According to the most generally accepted biogenetic theory[5] oxidative cyclisation of coclaurine (**7**) could give rise to crotsparine (**1**). Crotsparinine (**3**) could be formed by reduction of one of the double bonds of crotsparine (**1**) and sparsiflorine (**5**) would arise from crotsparine (**1**) by dienone-phenol rearrangement.

DL-2-[^{14}C]-Tyrosine was initially fed to young *C. sparsiflorus* plants and it was found that the plants were actively biosynthesizing crotsparine. In subsequent experiments, labelled hypothetical precursors were fed to the plants. The results of several feedings are recorded in Table 1; incorporation values are corrected for the obligatory loss

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[1] BHAKUNI, D S, SATISH, S and DHAR, M M (1970) *Phytochemistry* **9**, 2573, BHAKUNI, D S and DHAR, M M (1969) *Experientia* **25**, 354, BHAKUNI, D S. and DHAR, M M. (1968) *Experientia* **24**, 1026

[2] CHATTERJEL, A, MAJUMDAR, P L, MUKERJEE, R, SAHA, S K. and TALAPATRA, S K (1965) *Tetrahedron Letters* 1539

[3] DUBEY, M P, SRIMAL, R C and DHAWAN, B N (1969) *Indian J Pharmacol* **7**, 73

[4] BHAKUNI, D S, SATISH, S and DHAR, M M (1972) *Tetrahedron* **28**, 4679.

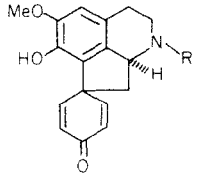
[5] BARTON, D H R and COHEN, T (1957) Festschrift A Stoll p 117 Birkhauser, Basel

Table 1 Tracer experiments on *Crotalaria sparsiflora*

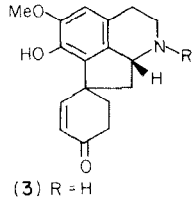
(±)-Precursor Fed	Alkaloids, incorporation % Crotsparine	Crotsparinine	Sparsiflorine
[2-^{14}C]-tyrosine	0.42	0.32	0.20
[3′,5′,8-^{3}H]-coclaurine	1.90	0.62	0.28
[3′,5′,8-^{3}H]-norcoclaurine	1.60	0.48	0.24
[3,5′,5-^{3}H]-isococlaurine	0.003	0.00	0.002

Incorporation corrected for loss of tritium

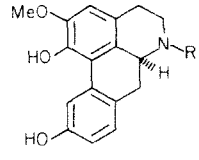
of tritium (±)-Coclaurine (**8**) was efficiently incorporated into crotsparine (**1**), crotsparinine (**3**) and sparsiflorine (**5**); (±)-norcoclaurine (**6**) was also incorporated into these alkaloids but with less efficiency As expected, labelled (±)-isococlaurine (**9**) was not incorporated into any of these alkaloids These negative results are in agreement with the classical theory[5] that cyclisation takes place only *para* and *ortho* to free phenolic hydroxyl groups Labelled crotsparine derived from coclaurine was converted into *N*-methylcrotsparine (**2**) which had the same molar activity Reduction to the tetrahydro derivative (**11**), treatment with hot methanolic NaOH furnished essentially radioinactive *N*-methyltetrahydrocrotsparine Thus all the tritium was, as expected, α- to the carbonyl group in crotsparine



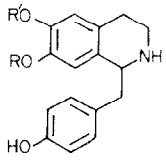
(**1**) R = H
(**2**) R = Me



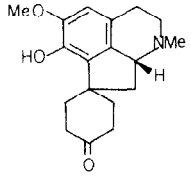
(**3**) R = H
(**4**) R = Me



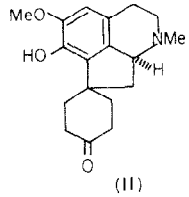
(**5**) R = H
(**6**) R = Me



(**7**) R′ = R = H
(**8**) R′ = Me, R = H
(**9**) R′ = H, R = Me



(**10**)



(**11**)

Labelled crotsparinine derived from coclaurine, was converted into *N*-methylcrotsparinine (**4**) having the same molar activity Reduction to the dihydro derivative (**10**), treatment with hot methanolic NaOH gave, essentially radio inactive *N*-methyldihydrocrotsparinine Thus all the tritium was α- to the carbonyl group in crotsparinine Labelled sparsiflorine derived from coclaurine, was converted into *N*-methylsparsiflorine (**6**) Base catalysed exchange reaction in water gave inactive *N*-methylsparsiflorine

The foregoing results thus established that crotsparine (**1**), crotsparinine (**3**) and sparsiflorine (**5**) are biosynthesized in *C. sparsiflorus* from coclaurine The presence of this key intermediate in *C. sparsiflorus* was confirmed by trapping experiment with DL-2-[^{14}C]-tyrosine when labelled coclaurine was isolated. Coclaurine thus fulfils both requirements of a true precursor

Crotsparine (**1**) and crotsparinine (**3**) have opposite configurations while sparsiflorine (**5**) and crotsparine (**1**) have the same configuration at the corresponding asymmetric centres. If crotsparine is a biosynthetic precursor of crotsparinine (**3**), a change of configuration should occur at position 6a in the benzylisoquinoline moiety of the proaporphine (**1**) during the course of biochemical transformations. Alternately crotsparine (**1**) and crotsparinine (**3**) could be biosynthesized by independent routes. Stuart and Grahm[6,7] have reported that in *Croton linearis* the proaporphine, crotonosine is derived from linearisine, a dihydroproaporphine having opposite configuration at the corresponding asymmetric centre. These workers[7] have recently mentioned that this conversion varies according to the sex of the plant However, these aspects of the biosynthesis of crotsparine and crotsparinine in *C. sparsiflorus* are being further investigated.

EXPERIMENTAL

Synthesis of benzylisoquinoline precursors The racemate of coclaurine,[8] norcoclaurine[9] and isococlaurine[10] were prepared by standard procedures

Labelling of precursors Tritium in the precursors was introduced by the technique published[11] earlier (±)-Coclaurine hydrochloride (120 mg) in T_2O (0 5 ml 200 mc) containing K *t*-butoxide (150 mg) was heated under N_2 (sealed tube) for 90 hr at 100° to give [3',5',8-^{3}H] coclaurine which was purified as the hydrochloride (60 mg) Dilution with inactive hydrochloride and repeated crystallization from MeOH–Et_2O did not cause loss of activity The other benzylisoquinoline precursors were tritiated in the same way

Feeding experiments The hydrochlorides of the labelled precursors were dissolved in H_2O Freshly cut young plants of *C sparsiflorus* were dipped into this aqueous solution pH *ca* 6 and the plants were allowed to take up the precursors Water was added to the container to wash the precursors and the plants were kept alive After a suitable period (4–5 days) for metabolism the plants were extracted for crotsparine, crotsparinine and sparsiflorine

Isolation of bases The plant material (typically 250–300 g wet wt) was macerated with MeOH (300 ml) and inactive crotsparine (150 mg) The MeOH was decanted and the residual plant material was percolated with fresh MeOH (6 × 300 ml) The combined MeOH extract was concentrated *in vacuo*, the green viscous mass thus obtained was treated with 2% aq tartaric acid (5 × 30 ml) The acidic solution was basified (pH 8) with NH_4OH and the base was extracted with $CHCl_3$ It was purified by chromatography and crystallization ($CHCl_3$–EtOAc) Radioisotopic purity was checked by preparing *N,O*-diacetylcrotsparine and *N*-methylcrotsparine Crotsparinine and sparsiflorine were similarly isolated Both the bases were purified by the procedure described earlier [1] Radioisotopic purity of these bases were checked by preparing *N,O*-diacetylcrotsparinine and *N*-methylsparsiflorine

Degradation of crotsparine Tritium labelled crotsparine derived from (±)-[3',5',8^{3}H]coclaurine was converted into *N*-methyltetrahydrocrotsparine (**11**) Treatment with refluxing 5% methanolic NaOH for 24 hr gave inactive *N*-methyltetrahydro-crotsparine

Degradation of crotsparinine Tritium labelled crotsparinine derived from (±)-[3',5',8-^{3}H]-coclaurine was converted into *N*-methyldihydrocrotsparinine (**10**) Treatment with refluxing 5% methanolic NaOH for 24 hr yielded inactive base

Degradation of sparsiflorine Tritium labelled sparsiflorine derived from (±)-[3',5',8-^{3}H]-coclaurine was converted into *N*-methylsparsisflorine (**6**) Treatment with refluxing 5% methanolic NaOH for 15 hr furnished inactive alkaloid

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