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THE BIOSYNTHESIS OF CULARINE*

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Key Word Index *Corydalis claviculata*; Fumariaceae; biosynthesis; (+)-sarcocapnidine; (+)-cularine; benzylisoquinoline alkaloids.

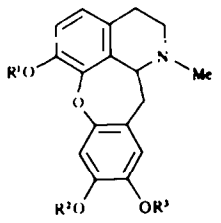
Abstract The incorporation of radioactive crassifoline into cularine proved crassifoline to be the precursor of the cularine alkaloids. Phenolic oxidative coupling is presented as the biogenetic mechanism.

INTRODUCTION

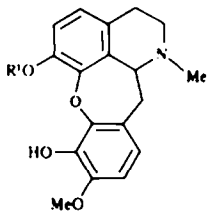
The cularines form a small group of benzylisoquinoline alkaloids. Because of their unusual skeleton incorporating an intramolecular diaryl ether, increasing attention has been focused on this class in the recent past [see 1 and references therein]. Three basic substitution patterns are known, each occurring in separate plant families and having a different biogenetic origin.

Trisubstituted cularines, such as the classical-type cularine (**1**), cularidine (**2**) and cularicine (**3**), as well as the isomeric sarcocapnidine (**4**) and claviculine (**5**), are limited to the Fumariaceae [1]. Both types have been assumed to arise by intramolecular phenolic oxidative coupling of a 7,8,3′,4′-oxygenated benzylisoquinoline [2]. The tetrasubstituted linaresine (**6**), isolated from a member of the Berberidaceae, may be biosynthesized from berberine via pseudobenzylisoquinolines [3]. Gouregine (**7**), on the other hand, occurring in the Annonaceae, could arise by oxidation of an aporphine alkaloid [4].

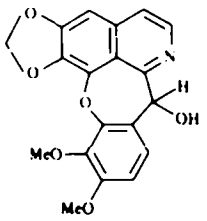
Corydalis claviculata DC. produces the cularine alkaloids **1**–**5** together with (+)-crassifoline (**8**), the first 7,8,3′,4′-substituted benzylisoquinoline isolated from a natural source and the hypothetical precursor of the cularines [5–8]. We now wish to report the incorporation of radioactive **8** into cularine (**1**) by feeding experiments, proving **8** to be the precursor of **1**. A preliminary report has already been published [9].



	R¹	R²	R³
1	Me	Me	Me
2	H	Me	Me
3	H	—CH₂—	
9	Me	Me	H



	R¹
4	Me
5	H



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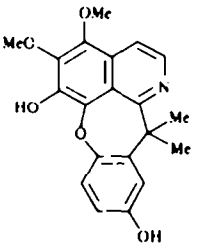

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RESULTS AND DISCUSSION

Natural (+)-crassifoline (**8**) was tritium labelled by Amersham International plc. The method used is known to exchange hydrogen in *ortho* and *para* positions to phenolic hydroxyl groups [10]. The purified product had a specific activity of 3.1 mCi/mg and a radiochemical purity of 95.7%. The labelling pattern was confirmed by bromination, which replaced the ^3H in *ortho* and *para* positions to phenolic functions [11] and gave an inactive product.

Five mCi of radioactive crassifoline (**8**) was applied to flowering *C. claviculata* plants by root uptake [12]. After feeding for 10 days the roots were washed with dilute acid and the plants lyophilized. The acid wash combined with residual nutrient solution was investigated for the conversion of **8** to cularine (**1**) by microorganisms adherent to the roots. No radioactive **1** could be detected.

Work-up of the plants yielded 31.6 mg of non-phenolic alkaloids with a total activity of 18.7 μCi. The phenolic bases, containing 170.1 μCi were not further investigated. The radioactivity of the non-phenolic alkaloids was located almost completely in cularine (**1**) as determined by thin layer radiochromatography. Compound **1** was separated from the other alkaloids by means of its insoluble oxalate salt and crystallized to a constant specific activity

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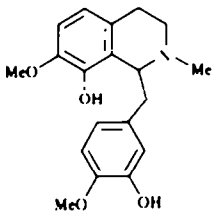
of 0.21 μCi/mg calculated as the cularine base. This corresponds to a [^{3}H]-retention of 0.32%. Considering the fact that one tritium is expelled during formation of the oxepine ring, this figure represents a minimum incorporation rate. The isolation of crassifoline (**8**) from *C. claviculata* and its incorporation into cularine (**1**) clearly demonstrates the key role of **8** as the precursor of the cularine alkaloids.

Direct phenolic oxidative coupling may be regarded as the biogenetic mechanism. The evidence so far accumulated concerning this mechanism is that two phenoxy radicals which exist largely with the unpaired electron on the oxygen are involved in the coupling reaction leading to C-O bond formation [13, 14]. Such is also very likely the case in the present instance since crassifoline (**8**) has the appropriate substitution pattern. Moreover, it possesses the correct arrangement of atoms at the chiral centre [6]. Thus, oxidation and *para* coupling of **8** would lead to compound **9**, which has not yet been isolated. Methylation would then furnish cularine (**1**). *Ortho* coupling of **8** gives rise to the isomeric derivatives **4** and **5**. This hypothesis is further supported by a biomimetic synthesis, demonstrating that only **8** could be coupled to **4** and **9** while the isomeric 4'-hydroxy-3'-methoxy-benzylisoquinoline produces only a pair of dienones which could not be rearranged to **4** or **9** [15, 16]. Interestingly, the classical 3',4'-oxygenated cularines are formed predominantly. Thus, the major alkaloids cularine (**1**), cularidine (**2**) and cularicine (**3**) represent *ca* 63% of the total alkaloidal fraction of *C. claviculata*, while the isomeric 4',5'-substituted derivatives sarcocapnidine (**4**) and claviculine (**5**) form only 3.7% [6]. This may be caused by steric hindrance.

Stylopine (**10**) and protopine (**11**) are also present in *C. claviculata* [5, 6]. Neither contained any radioactivity after feeding crassifoline (**8**). These alkaloids have been shown to be biosynthesized from (+)-reticuline (**12**) [17, 18]. This pathway seems to be reasonable in the present instance too, because **12** as well as other intermediates of this pathway (i.e. scoulerine and cheilanthifoline) were isolated from *C. claviculata* [6].



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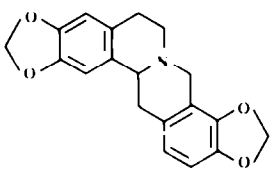

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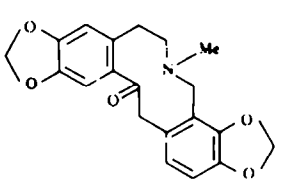

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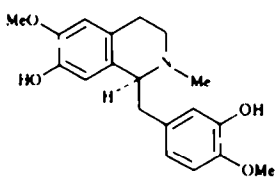

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Thus, two different biogenetic pathways proceeding from isomeric benzylisoquinolines have to be considered for *C. claviculata*: the 7,8-oxygenated crassifoline (**8**) is the precursor of the cularine alkaloids, while some of the other type bases are most likely derived from the 6,7-substituted reticuline (**12**).

EXPERIMENTAL

Mps are uncorr. Radioactivity of alkaloid extracts and determination of radiochemical purity was monitored by analytical radio-TLC. Liquid scintillation counting with [^{226}Ra] as external standard was used to determine the radioactivity of isolated compounds. An analogous treated inactive probe served as background.

Plant material. C. claviculata plants were collected from a natural location near Bremen, West Germany, in summer 1983. The roots were carefully washed and placed in darkened 25 ml flasks with a nutrient soln.

Preparation of ^{3}H-*crassifoline* (**8**). *Ca* 20 mg of **8** were labelled by Amersham International plc, U.K., by heating with ^3H_2O. Labile ^{3}H was removed by evaporating × 3 with EtOH; total act. of the product 121 mCi; radiochemical purity 69.2%. CC of 4 mg of ^{3}H-**8** on silica gel with CH_2Cl_2–MeOH (9:1) gave 3.2 mg of 95.7% pure **8**; sp. act. 3.1 mCi/mg.

Bromination of **8**. ^{3}H-**8** (0.3 mg; total act. 0.93 mCi) dissolved in 2 ml $CHCl_3$ was stirred with 8 mg Br_2 in 3 ml $CHCl_3$ for 48 hr at room temp. After addition of 5 drops of 5% Na_2SO_3 soln and gentle heating, the aq. layer was brought to pH ~ 8 and the organic layer separated. The aq. soln was extracted × 3 with 5 ml $CHCl_3$, the combined organic solvents dried over Na_2CO_3 and evaporated under red. pres. to yield a product with a total act. of 2.8 μCi (0.3% of the original act.).

Feeding experiments. Compound **8** (1.6 mg; 5 mCi; 3.1 mCi/mg) was dissolved in 1 ml 0.1% H_3PO_4, diluted with H_2O to 10.0 ml and added in aliquots to five flowering plants. Nutrient soln was added as uptake demanded. After 10 days the roots were washed with 0.01 N H_2SO_4 and the plants lyophilized. The acid wash combined with residual nutrient soln was brought to pH ~ 8 and extracted × 3 with 100 ml CH_2Cl_2. After drying over Na_2CO_3 the organic solvent was removed under red. pres. and the residue dissolved in 5 ml CH_2Cl_2. Radioactivity recovered: 489 μCi; no cularine (**1**) could be detected by radio-TLC.

Isolation of ^{3}H-*cularine* (**1**). Work-up of the plant material in the usual manner [6] yielded 56.2 mg phenolic bases (total act. 170.1 μCi) and 31.8 mg non-phenolic alkaloids (total act. 18.7 μCi). Radio-TLC of the latter in different solvent systems showed that ^{3}H-**1** accounted for 95% of the radioactivity. Stylopine (**10**) and protopine (**11**) were essentially inactive. 6.3 mg of the non-phenolic extract was crystallized from MeOH with 15.5 mg of inactive cularine oxalate to a constant sp. act. of 0.17 μCi/mg (0.21 μCi/mg calc. as cularine base); incorporation rate 0.32%. Mp (^{3}H-**1**): 243° (lit. [5]: 245°); Mmp 243°C; identical R_f-values as natural **1**.

Isolation of stylopine (**10**) *and protopine* (**11**). The non-phenolic extract (4 mg) (2.4 μCi) was subjected to prep. TLC ($CHCl_3$–EtOAc–MeOH, 2:2:1) and the zones corresponding to **10** (R_f 0.70) and **11** (R_f 0.18) eluted (CH_2Cl_2–MeOH, 9:1) and assayed for radioactivity. No radioactivity could be detected.

REFERENCES

1. Gözler, B. and Shamma, M. (1984) *J. Nat. Prod.* **47**, 753.
2. Abu Zarga, M. H., Miana, G. A. and Shamma, M. (1981)

Tetrahedron Letters **22**, 541.
3. Firdous, S., Freyer, A. J. and Shamma, M. (1984) *J. Am. Chem. Soc.* **106**, 6099.
4. Leboeuf, M., Cortes, D., Hocquemiller, R., Cavé, A., Chiaroni, A. and Riche, G. (1982) *Tetrahedron* **38**, 2889.
5. Manske, R. H. F. (1940) *Can. J. Res.* **18B**, 97.
6. Blaschke, G. and Scriba, G. (1985) *Phytochemistry* **24**, 585.
7. Boente, J. M., Castedo, L., Cuadros, R., Rodriguez de Lera, A., Saa, J. M., Suau, R. and Vidal, M. C. (1983) *Tetrahedron Letters* **24**, 2303.
8. Guinaudeau, H. and Allais, D. P. (1984) *Heterocycles* **22**, 107.
9. Blaschke, G. and Scriba, G. (1983) *Tetrahedron Letters* **24**, 5855.
10. Kirby, G. W. and Ogunkoya, L. (1965) *J. Chem. Soc.* 6914.
11. Barton, D. H. R., Kirby, A. J. and Kirby, G. W. (1965) *Chem. Commun.* 52.
12. Stermitz, F. R. and Rapoport, H. (1961) *J. Am. Chem. Soc.* **83**, 4045.
13. Dyke, S. F. (1977) *Heterocycles* **6**, 1441.
14. Waters, W. A. (1971) *J. Chem. Soc. B* 2026.
15. Kametani, T., Fukumoto, F. and Fujihara, M. (1971) *Bioorg. Chem.* **1**, 40.
16. Birch, A. J., Jackson, A. H., Shannon, P. V. R. and Stewart, G. W. (1975) *J. Chem. Soc. Perkin Trans.* 1, 2492.
17. Battersby, A. R., Staunton, J., Wiltshire, H. R., Francis, R. J. and Southgate, R. (1975) *J. Chem. Soc. Perkin Trans.* 1, 1147.
18. Takao, N., Iwasa, K., Kamigauchi, M. and Sugiura, M. (1976) *Chem. Pharm. Bull.* **24**, 2859.