Tetrahedron Vol 39, No 4, pp 577 to 580, 1983
Printed in Great Britain

0040-4020/83/040577-04$03.00/0

EGENINE: A POSSIBLE INTERMEDIATE IN PHTHALIDEISOQUINOLINE BIOGENESIS

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(*Received in U.S.A. 20 September 1982*)

Abstract: (+)-Egenine (4), the first phthalideisoquinoline hemiacetal isolated from a natural source has been found in Fumaria vaillantii Loisel. (Fumariaceae). (+)-Egenine may act as the immediate biogenetic precursor of the accompanying alkaloid (+)-bicuculline (1).

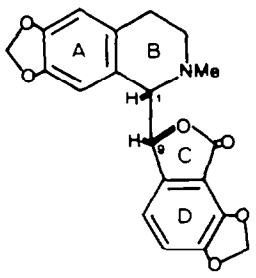
The phthalideisoquinolines represent one of the most important subgroups among the isoquinoline alkaloids. More than 25 naturally occurring phthalideisoquinolines of the classical type are known. They all possess a tetracyclic skeleton and an intact ring B as represented typically by the alkaloid (+)-bicuculline (1).[3,4]

The biogenesis of the phthalideisoquinolines poses an interesting problem. In one of the key *in vivo* experiments, the doubly labeled berbine (-)-scoulerine (2) was fed to Papaver somniferum L. (Papaveraceae). When the curtain lifted and the radioactive products were analyzed, it was found that the labels were carried by the phthalideisoquinoline (-)-α-narcotine (3) as indicated below. Only a minor loss of the tritium label was observed. It follows that berbines are precursors for the phthalideisoquinolines in nature in such a manner that the C-14 asymmetric center in the berbine maintains its integrity.[5] What remained unknown was the exact sequence of steps involved in the conversion of a berbine into a phthalideisoquinoline. Clearly, a series of intermediates must be involved in this transformation, all of which must be quite transitory and produced in small amounts. This is underlined by the fact that the very large number of alkaloid isolation studies carried out in the past had yielded only berbines and classical phthalideisoquinolines, with no indication of any intermediates in the sequence.

We now describe the isolation and structural elucidation of the alkaloid (+)-egenine (4), the first phthalideisoquinoline hemiacetal isolated from a natural source. This compound may throw some light on the final step involved in the conversion of a berbine into a phthalideisoquinoline of the classical type.

The dried and powdered aerial parts of Fumaria vaillantii Loisel. (Fumariaceae) (650 g), collected near Simav, Kütahya, in western Anatolia, were extracted with ethanol at room temperature. The crude, basic, alkaloidal fraction was subjected to repeated chromatography to provide 1.6 mg of amorphous (+)-egenine (4), $C_{20}H_{19}O_6N$.

The 200 MHz FT NMR spectrum of egenine in deuteriochloroform has been summarized around expression 4. Obvious structural features revealed are an N-methyl singlet at δ 2.54, two methylenedioxy substituents near δ 6.00, two aromatic protons appearing as singlets at δ 6.36 and 6.78, and two additional adjacent aromatic protons responsible for a doublet of doublet pattern centered at δ 6.58 with J_o 7.9 Hz. A one-proton doublet at δ 3.93 can readily be assigned to H-1 by comparison with the NMR spectra of phthalideisoquinolines.[3] Another one-proton doublet at δ 5.44 must repre-



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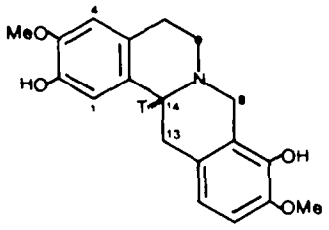

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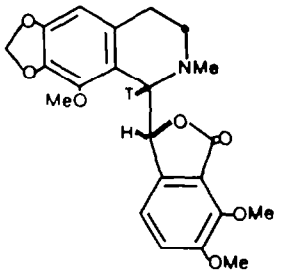

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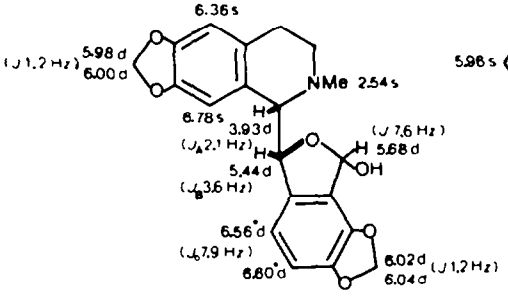

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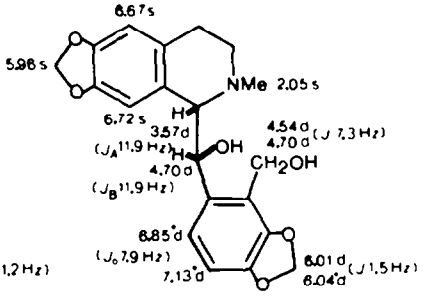

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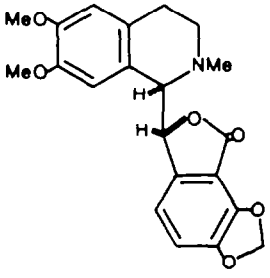

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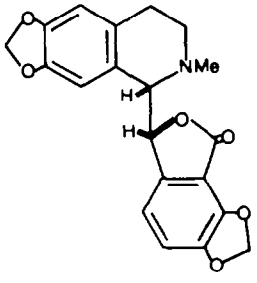

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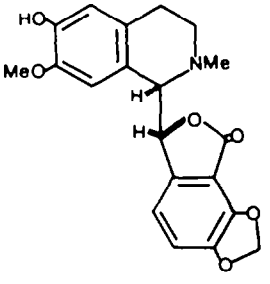

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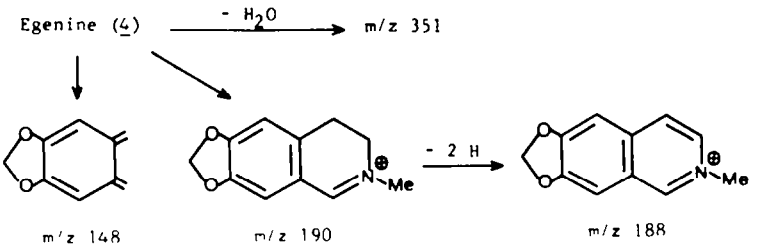
sent H-9 by analogy with the chemical shift of the same proton in the known phthalideisoquinolines, which usually falls between δ 5.48 and 5.60.[3] Finally, a one-proton doublet at δ 5.68 could be assigned to a hemiacetal linkage as shown in expression 4.[6]

The electron impact mass spectrum of egenine does not show a molecular ion. Instead, there is a small m/z 351 peak representing $(M - 18)^+$. The base peak m/z 190 results from the familiar benzylic cleavage of phthalideisoquinolines. Other important peaks are m/z 188 and 148.

To ascertain the molecular weight, a chemical ionization mass spectrum of the alkaloid was obtained using isobutane. This spectrum showed an m/z 370 peak, corresponding to a molecular weight of 369 calculated for the composition $C_{20}H_{19}O_6N$.

The scheme below summarizes some of the mass spectral findings.



The IR spectrum of egenine does not show any carbonyl absorption. The UV spectrum, with a maximum at 290 nm, is characteristic of tetrahydrobenzylisoquinolines or berbines bearing two methylenedioxy substituents. It also confirmed the absence of a conjugated γ-lactone ring since classical type phthalideisoquinolines such as 1 show maxima near 296 and 320 nm mirroring the extensive conjugation present.[3]

It is known that all classical phthalideisoquinolines which encompass the S configuration at C-1, such as (+)-bicuculline (1), exhibit a positive specific rotation.[3] Since egenine shows $[\alpha]_D^{23}$ +214° (c 0.11, $CHCl_3$) or $[\alpha]_D^{23}$ +99° (c 0.12, MeOH), it was suspected that it too possesses the S chirality at C-1.

A report is extant in the literature of the partial reduction of the lactone carbonyl of classical phthalideisoquinolines to supply the corresponding hemiacetals.[7] In our hands, however, low temperature reduction of the limited amount of (+)-bicuculline (1) in our possession, using either sodium borohydride in ethanol, or redal in ether or toluene, or yet dibal in methylene chloride, provided only (-)-bicucullinediol (5).[8] Final proof for the structure of egenine was then obtained when sodium borohydride in ethanol reduction of (+)-egenine (4) provided a diol chromatographically and spectrally (MS, UV, IR, NMR, CD) identical with (-)-bicucullinediol (5).

The isolation and characterization of (+)-egenine (4) may fill a significant gap in the succession of relays involved in phthalideisoquinoline biogenesis. It is possible to envision an in vivo process in which a berbine alkaloid related to 2 is oxidized in stages at C-8 and C-13, and is also N-methylated to produce (+)-egenine (4) or one of its close analogs. Enzymic oxidation of 4 would then generate (+)-bicuculline (1) which, very significantly co-occurs with (+)-egenine in F. vaillantii Loisel. Other classical type phthalideisoquinolines we have encountered in the plant are (-)-adlumine (6), (-)-capnoidine (7) and (-)-corledine (8).

Acknowledgment: This research was supported by grant CA-11450 from the National Cancer Institute, National Institutes of Health, USDHHS.

Experimental

Isolation and Purification: The crude, basic, alkaloidal fraction was fractionated on a column of silica gel (70-230 mesh) using chloroform as eluant, and then increasing amounts of methanol in chloroform. Fractions eluted with 2, 3, and 5% methanol in chloroform were combined and refractionated on a column of Merck Silica Gel H (for TLC) using chloroform-methanol-ammonium hydroxide (98:2:0.5 v/v). Fractions of 3 mL were collected. Combined fractions 23-26 were subjected to preparative TLC on Merck Silica Gel G analytical glass plates using the solvent system benzene-chloroform-acetone-methanol (40:30:15:10). The egenine band, R_f 0.54, was further purified by TLC in the same system.

(+)-Egenine (4), λ max MeOH 212, 233 sh, 290 nm (log ε 4.11, 3.75, 3.71), no change upon addition of hydroxide base; ν max $CHCl_3$

Redal = sodium bis(2-methoxyethoxy)aluminum hydride; dibal = diisobutylaluminum hydride.

3350, 2900, 1605, 1500, 1480, 1465, 1385, 1295, 1240, 1100, 1035, 1000, 970, 925 900, 865, 835 cm^{-1}; CD $\Delta\varepsilon$(nm) MeOH +1.5(293), +0.1(260), +5.1(232) with positive tail beyond 232 nm; ei ms m/z 351 (0.3), 336 (0.2), 320 (0.4), 192 (2), 191 (19), 190 (100), 188 (28), 178 (5), 175 (4), 162 (21), 149 (14), 148 (18).

Reduction of (+)-Egenine (4): The alkaloid (1.0 mg) in 5 mL ethanol was treated with excess sodium borohydride and stirred overnight at room temperature. The solvent was evaporated, excess sodium borohydride destroyed by dropwise addition of 1% hydrochloric acid, and the solution made alkaline with ammonium hydroxide. Following extraction with chloroform, the organic layer was dried over anhydrous sodium sulfate and evaporated to give 1 mg of 5. Essentially the same procedure was followed in the reduction of (+)-bicuculline (1) to afford the same product 5.

(-)-Bicucullinediol (5), $C_{20}H_{21}O_6N$, TLC R_f 0.27 in system described above, $[\alpha]_D^{23}$ -17° (c 0.15, $CHCl_3$); λ max MeOH 210, 237 sh, 291 nm (log ε 4.40, 3.83, 3.81); ν max $CHCl_3$ 3010, 3000, 2940, 2860, 1600, 1480, 1455, 1380, 1340, 1260-1190, 1110, 1040, 1020, 930 cm^{-1}; CD $\Delta\varepsilon$(nm) MeOH -3.1(297), -0.5(264), -2.5(246), +2.1(231) with negative tail beyond 231 nm; ei ms m/z 354 (M - 17) (0.4), 353 (0.4), 322 (0.4), 192 (2), 190 (100), 188 (4), 163 (2), 149 (5), 148 (4).

(+)-Bicuculline (1), (-)-adlumine (6), (-)-capnoidine (7) and (-)-corledine (8): The fractions eluted from the silica gel column using chloroform-methanol were further purified by TLC to provide (+)-bicuculline (16 mg), (-)-adlumine (110 mg), (-)-capnoidine (30 mg), and (-)-corledine (2 mg). These materials were spectrally and chromatographically identical with authentic samples.

References and Footnotes

1. Permanent address: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ege University, Izmir, Turkey.
2. Permanent address: Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir, Turkey.
3. For a complete listing of the phthalideisoquinoline alkaloids, together with their physical and spectral properties, see G. Blaskó, D.J. Gula and M. Shamma, J. Nat. Prod., 45, 105 (1982).
4. The term "classical" refers to phthalideisoquinolines with the intact tetracyclic skeleton, including the γ-lactone ring. It thus helps to differentiate such alkaloids from the secophthalideisoquinolines in which ring B has been cleaved.
5. A.R. Battersby, M. Hirst, D.J. McCaldin, R. Southgate and J. Staunton, J. Chem. Soc., C, 2163 (1968).
6. The one-proton absorption at δ 5.68 is a doublet due to vicinal coupling with the alcoholic proton, see E. Pretsch, T. Clerc, J. Seibl and W. Simon, "Tabellen zur Strukturaufklärung Organischer Verbindungen", Springer Verlag, Heidelberg (1976), p. H60.
7. H. Schmidhammer, Sci. Pharm., 49, 304 (1981).
8. G. Nonaka and I. Nishioka, Chem. Pharm. Bull., 23, 294 (1975).
9. We consider it unlikely that (+)-egenine (4) is formed biogenetically from (+)-bicuculline (1). Rather, a classical phthalideisoquinoline such as 1 would tend to N-methylate and undergo Hofmann elimination to provide the secophthalideisoquinoline aobamidine. This alkaloid would then suffer oxidation to fumariflorine; see Ref. 3 above.