Structure of Fumarophycine

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Fumarophycine, an alkaloid derived from *Fumaria officinalis* of Bulgarian origin, is shown by spectroscopic methods to be a new member of the spiroisoquinoline group. It has been related by chemical methods to the alkaloid, fumaritine, of known constitution.

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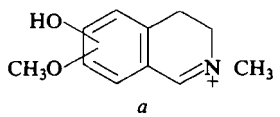
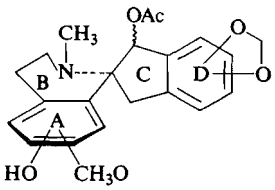
The alkaloids of *Fumaria officinalis* were first examined by Manske (1) in 1938. More recently he has reported (2) the isolation of two other minor alkaloids from this source. Alkaloids belonging to the protoberberine (1), protopine (1), and the spiroisoquinoline (3–5) groups are now known to be elaborated by this species. A few years ago Mollov *et al.* (6) examined *F. officinalis* of Bulgarian origin from which fumarophycine, **1**, was isolated as the major alkaloid along with smaller amounts of protopine, cryptopine, and sinactine. They assigned to fumarophycine the formula, $C_{22}H_{23}NO_6$, and from its properties inferred that it was a new alkaloid. By p.m.r. spectroscopy, it was shown that fumarophycine had a methylenedioxy group, an *O*-methyl group, a phenolic hydroxyl group, an alcoholic *O*-acetyl group, an *N*-methyl group and four aromatic protons. Alkaline hydrolysis of fumarophycine gave a compound of the expected mass while methylation with diazomethane gave *O*-methylfumarophycine thus confirming the character of the *O*-acetyl and hydroxyl functions. Further examination of fumarophycine using high resolution mass spectrometry and 100 MHz p.m.r. spectroscopy has enabled us to assign its structure.

The mass spectrum of fumarophycine provided the first clue that it was a new representative of the spiroisoquinoline group of alkaloids. In the high mass region besides a molecular ion peak at *m/e* 397 (25)[1] there were peaks at 355 (26), M-C_2H_2O, 354 (100), M-C_2H_3O, and 337 (48), M-$C_2H_4O_2$. The most significant peak in the spectrum appeared at *m/e* 192 (6) of composition $C_{11}H_{14}NO_2$. Previous examination (5, 7) of the mass spectra of spiroisoquinoline alkaloids containing a hydroxyl group in the spiro system has shown that they fragment to dihydroisoquinolinium ions and these ions are often the most intense in the spectra. The low intensity of this ion, to which we assign structure *a*, in the case of fumarophycine, is not surprising since the hydroxyl group is acetylated. Fragmentation to give *m/e* 192 could occur only after loss of ketene. Thus from its mass spectrum and from its origin it appears that fumarophycine is another example of the spiroisoquinoline group of alkaloids as represented in partial structure *b*. The methoxyl and hydroxyl groups are placed in ring A because of the composition of ion *a*. The mass spectrum does not, however, provide any information concerning the substitution pattern. It was necessary, therefore, to turn to p.m.r. spectroscopy which has been used so successfully with other members of this series (3–5, 7, 8) to confirm the structure and to define the substitution pattern and the relative stereochemistry.



a



b

[1]Ion intensities are given in brackets. The composition of the ions has been established by accurate mass measurement.

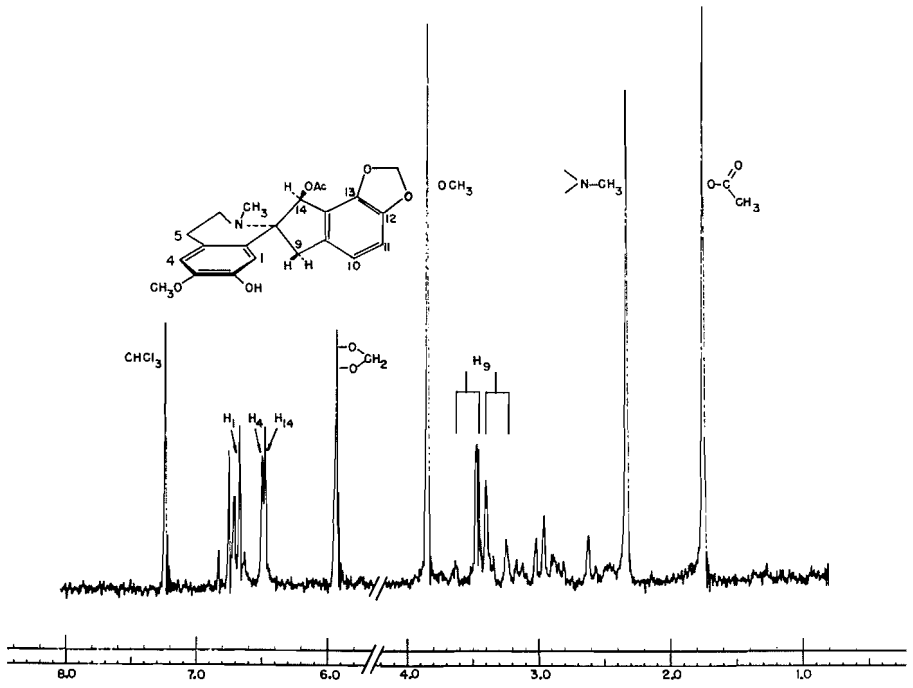


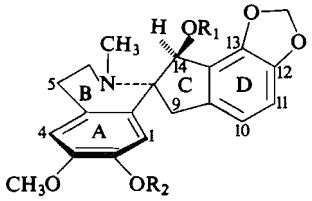
FIG. 1. The 100 MHz p.m.r. spectrum of fumarophycine.

The 100 MHz p.m.r. spectrum of fumarophycine, Fig. 1, has confirmed the original assignment of substituent groups made by Mollov *et al.* Thus, we find a signal of area 2 at 5.94 δ assigned to the methylenedioxy group and three singlets of area 3 at 1.74, 2.32, and 3.94 which are assigned, respectively, to acetyl-methyl, *N*-methyl and *O*-methyl groups. In the region 2.32 to 3.94 δ there are signals corresponding to six aliphatic protons. Two of these are present as an AB quartet, δ H_A = 3.32, H_B = 3.55, J_{AB} = 16 Hz. These signals are assigned to the benzylic protons at C-9. The remaining four protons in this region may be attributed to those at C-5 and -6. In the region 6.0–7.0 δ there are signals corresponding in area to five protons. Two of these are present as an aromatic AB quartet, δ H_{10} = 6.66, H_{11} = 6.79, $J_{H_{10}H_{11}}$ = 8.0 Hz while the other three are singlets at 6.67, 6.50 and 6.48 δ representing the protons at C-1, -4, and -14 respectively. The assignment of these signals to specific protons in the system is based on measurement of nuclear Overhauser effects (n.O.e.) (9).

The n.O.e. measurements were used in the elucidation of the structure of other alkaloids of this ring system (4, 5, 7, 8). When this technique was applied to **1**, the following results were obtained. Irradiation of the signal at 2.32 δ (*N*-CH_3) resulted in an increase of area of 25% for the signal at 6.48 δ. Accordingly, we can assign the latter signal to the proton geminal to the acetoxy group at C-14 and at the same time we have established its relative stereochemistry. Irradiation at the center of the benzylic AB quartet increased the area of the signal at 6.67 δ by 25% which permits assignment of this singlet to the proton at C-1. The singlet at 6.50 δ must then be attributed to C-4. This has been confirmed by the observation that there is an increase in area of 8% for this signal on irradiation of the axial proton at C-5 (3.50 δ). There is no observable change in area of the signal at 6.67 δ as a result of this latter irradiation. The assignment of the methoxyl group at C-3 is based on the evidence that irradiation of the methoxyl signal at 3.94 δ increases the area of the signal at 6.50 δ by 24% but does not affect the signal at 6.67 δ. Finally we are able to assign the protons of the aromatic AB quartet to C-10 and -11. Irradiation of the

protons of the benzylic AB quartet increased the area of the signal at 6.66 δ by 20%. This is compatible with the observation that the higher field doublet of this AB quartet is broader than its lower field counterpart, a reflection of the fact that ortho-benzylic coupling is stronger than meta (10).

The presence of the acetyl group in fumarophycine has deshielded the proton at C-1 relative to non-acetylated analogues (5). Moreover, the relatively high field absorption of the acetyl-methyl suggests that it is shielded by one or other of the aromatic rings (probably ring A) of this system. Similar effects have been observed in the spectrum of diacetyl ochrobirine (8).



1 R_1 = Ac; R_2 = H
2 R_1 = H; R_2 = H
3 R_1 = Ac; R_2 = Ac
4 R_1 = H; R_2 = CH_3

The p.m.r. data are thus compatible with the spiroisoquinoline structure and indeed demonstrate that **1** is the only satisfactory formulation of fumarophycine. It is, therefore, a derivative of fumaritine **2** (5) whose structure has been confirmed by synthesis (11). We were able to establish a direct relationship by converting both **1** and **2** by acetylation to the common derivative **3**. The i.r. and p.m.r. spectra of **3** prepared from **1** and **2** were identical in all respects.

One feature of the chemistry of fumarophycine remains unresolved. In their initial report Mollov *et al.* (6) reported on the alkaline hydrolysis of fumarophycine to fumarophycinol, m.p. 128–30°, and its subsequent methylation with diazomethane to *O*-methylfumarophycinol, m.p. 134–137°. According to the structure which has been established for fumarophycine, fumarophycinol would be expected to be identical with fumaritine and *O*-methylfumarophycinol with fumaricine (**4**) but they were not. For each pair the melting points were different, the mass spectra were virtually identical, but the p.m.r. spectra, although similar, showed minor but distinct differences. The reason for these differences has not been established and their investigation must await the availability of further supplies of the alkaloid.

Experimental

Methods

Mass spectra were recorded on a CEC 21-110B double-focusing mass spectrometer. For high resolution work spectra were recorded on plates and accurate mass measurements were made using perfluorokerosene as a marker (12).

The p.m.r. spectrum was recorded using the frequency sweep mode of a Varian HA-100 spectrometer. Samples were dissolved in $CDCl_3$ using added TMS as the internal locking signal. Chemical shifts were measured relative to TMS using a V4315 frequency counter incorporated in the instrument. Double irradiation was achieved by employing a Hewlett–Packard 201C audiogenerator at the desired frequency.

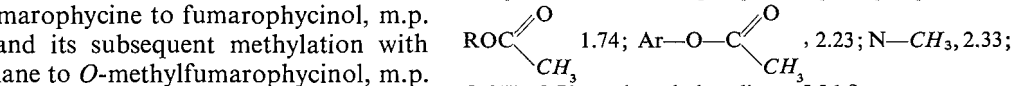
Fumarophycine

The isolation and properties of this compound have been described (6). The molecular formula of fumarophycine has been confirmed by high resolution mass spectrometry.

Mol. Wt. Calcd. for $C_{22}H_{23}NO_6$: 397.153. Found: 397.154.

O-Acetylfumarophycine

Fumarophycine (10 mg) was dissolved in acetic anhydride (5 ml) containing pyridine (0.1 ml) and the mixture was allowed to stand at room temperature for 24 h. The excess anhydride and pyridine were evaporated under reduced pressure, the residue triturated in aqueous sodium carbonate, and the suspension extracted several times with chloroform. The chloroform extract was dried over sodium sulfate, and evaporated to a residue which was purified by preparative t.l.c. on silica gel using $CHCl_3$:MeOH = 99:1 to develop the plate. The residue obtained upon elution could not be induced to crystallize but on an analytical thin layer plate it showed only a single spot. The mass spectrum showed a molecular ion at *m/e* 439. The i.r. spectrum had two bands in the carbonyl region at 1730 and 1760 cm^{-1} attributed to alcoholic *O*-acetate and phenolic *O*-acetate groups, respectively. The p.m.r. spectrum of **2** is similar to that of **1** but shows a new peak attributed to the methyl of the phenolic-*O*-acetate group. The principal peaks are



OCH_3, 3.79; and methylenedioxy, 5.96 δ.

O,O-Diacetylfumaritine

Fumaritine was acetylated in the same manner as fumarophycine and the product worked up and purified as described above. Its properties were identical in all respects with those of *O*-acetylfumarophycine.

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