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STRUCTURES OF MARCFORTINE B AND C (X-RAY ANALYSIS), ALKALOIDS FROM PENICILLIUM ROQUEFORTI[§]

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Summary : Marcfortine B and C are minor alkaloids isolated from the mycelium of Penicillium roqueforti. The structure of marcfortine B was established by spectral means and that of marcfortine C by X-ray diffraction analysis.

A previous study [1] of the Penicillium roqueforti strain B26 resulted in the isolation, along with roquefortine [2], of three new alkaloids which have been designated marcfortine A, B and C. The major component, marcfortine A, $C_{28}H_{35}N_3O_4$, was shown to have structure 1 and to be the first fungal alkaloid to possess a seven membered ring formed by linkage of a isoprene unit to two phenolic hydroxy-groups on the tryptophan unit. We herein report the structural elucidation of the two other components, marcfortine B 2 and marcfortine C 3.

Marcfortine B 2 crystallized from ethyl acetate, m.p. 178-180°, $[\alpha]_D^{22}$ - 67.7° (c 1.77, $CHCl_3$) ; u.v. λ_{max} 229 nm (ε 20755). The high resolution mass spectrum established the molecular formula of 2 as $C_{27}H_{33}N_3O_4$ ($M^{+\cdot}$ at m/z 463.2479) which differed from that of marcfortine A by one methylene unit. This difference is accounted for by the absence of a resonance due to a $N-CH_3$ grouping in the ^1H-n.m.r. spectrum of 2 and suggests that 2 was 29-nor-marcfortine A. The 250 MHz ^1H n.m.r. of marcfortine B (Table I) displayed in the downfield region the same resonances as that of 1, namely the AB quartet due to the two contiguous aromatic protons (H-4 and H-5) and the widely separated olefinic AB quartet due to H-24 and H-25. The chemical shifts for the four methyl groups were also identical whereas the pattern between δ 1.5 and 3.7 ppm was slightly different. Double resonance experiments identified other signals : the triplet due to H-20 and the three two-proton AB patterns due to the methylenes α to nitrogen (H-12 and H-17) and to the C-10 methylene. The ^{13}C n.m.r. spectrum (Table II) of marcfortine B 2 was also

[§]This paper is dedicated to the memory of the late Professor F. Šorm.

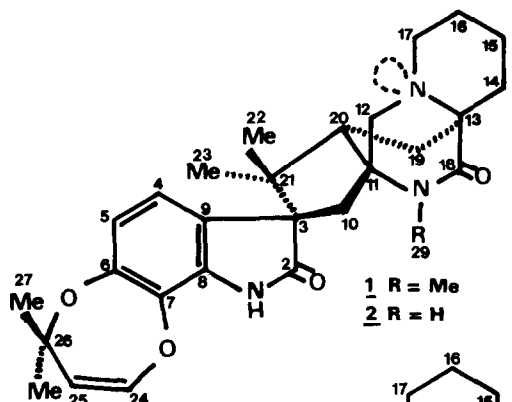
Table I : 250 MHz ^{1}H n.m.r. spectra of 2 and 3 [δ in ppm, J as (Hz)].

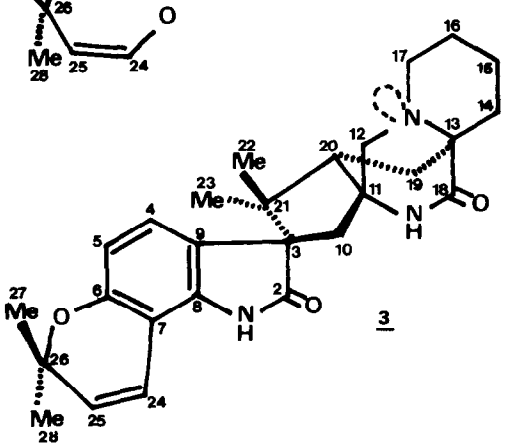
	2	3
H-4	6.66 d (8.1)	6.86 d (8.1)
H-5	6.79 d	6.42 d
H-24	6.39 d (7.5)	6.44 d (10)
H-25	4.91 d	5.82 d
H-12	3.71 (11.2)	3.68 d (11.2)
	2.48 br.d	2.45 br.d
H-20	3.08 t (10)	3.08 t (10)
H-17	2.70 dd (11.2)	2.67 dd (11.2)
	2.48 br.d	2.45 br.d
26-Me	1.45	1.45
	1.43	1.43
21-Me	1.08	1.08
	0.81	0.81

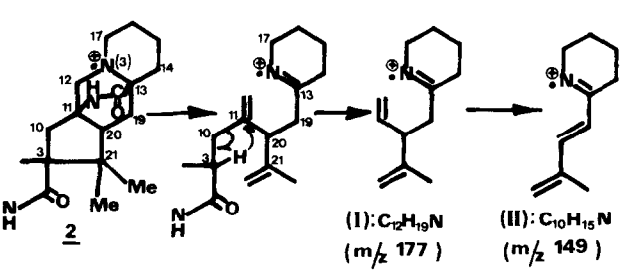
Table II : ^{13}C n.m.r. spectra [3] of 1, 2 and 3; a-d Signals may be reversed.

	1		2	3 ※
C-2	183.3	s	183.1	185.5
C-3	60.6	s	61.4	61.0
C-4	120.2	d	120.4	124.4
C-5	114.9[a]	d	115.0[a]	109.4
C-6	146.2	s	146.1	153.1
C-7	132.8[b]	s	132.6[b]	105.6
C-8	135.4[b]		135.4[b]	137.8
C-9	124.9	s	124.9	121.2
C-10	37.0	t	39.9	39.6
C-11	63.1[c]	s	61.4[c]	61.2
C-12	61.5	t	61.4	61.4
C-13	64.2[c]	s	63.1[c]	62.6
C-14	31.7	t	31.1[d]	30.8
C-15	20.7	t	20.9	20.7
C-16	25.9	t	25.9	25.7
C-17	54.5	t	54.6	54.5
C-18	173.9	s	177.1	177.2
C-19	31.7	t	31.8[d]	31.5
C-20	52.9	s	54.2	54.3
C-21	46.5	t	46.7	46.3
C-24	139.2	d	139.1	116.3
C-25	117.2[a]	d	117.8[a]	131.4
C-26	79.8	s	79.8	76.8
26-Me	29.9 ; 29.9	q	30.1, 29.7	27.8, 27.8
21-Me	20.7 ; 23.8	q	23.9, 20.6	23.7, 20.1
N-Me	26.4	q		

※ No Off Res.

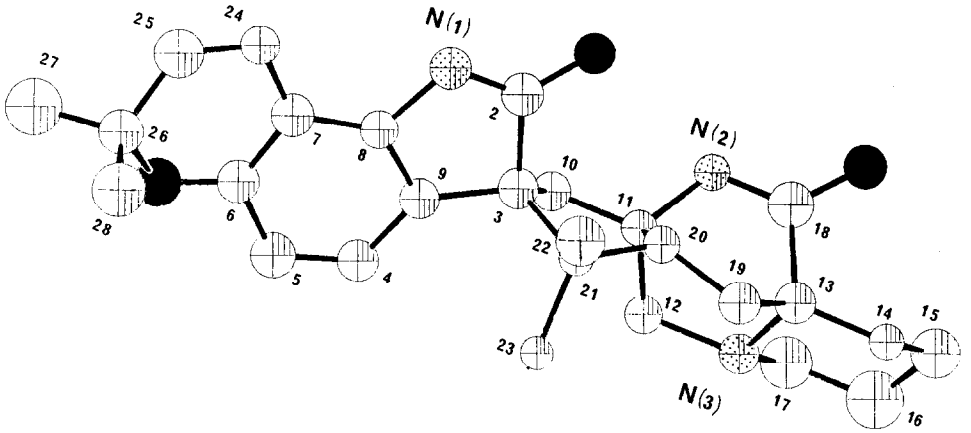






Scheme 1

closely related to that of 1 and showed the presence of an indolinone (δ 183.1) and of an amide function (δ 177.1). The structure of 2 was further supported by its mass spectrum which showed abundant fragments ions at m/z 420, due to the loss of NHCO, and at m/z 177.1480 ($C_{12}H_{19}N$, 29 %) and at m/z 149.1195 ($C_{10}H_{15}N$, 100 %) which can be attributed to ions (I) and (II), respectively (scheme 1). These ions probably arise by cleavage of the 3,21 carbon bond via a McLafferty rearrangement, loss of the elements of fulminic acid (HNCO) and fragmentation mainly directed by the charge-stabilizing nitrogen atom [N(3)].



Figure

Molecular structure of marcfortine C

Marcfortine C 3 crystallized from ethylacetate as colorless prisms, m.p. 264-267°, $[\alpha]_D^{22}$ - 64.4° (c 1.1, $CHCl_3$) ; u.v. λ_{max} 246 nm (ε 11490). It has the composition $C_{27}H_{33}N_3O_3$ ($M^{+\cdot}$ 447) which in conjunction with its ^1H-n.m.r. spectrum (Table I) indicated that 3 differs from marcfortine B 2 by the presence of singly oxygenated six membered ring located on the tryptophan moiety. The complete structure of marcfortine C 3 was provided by single crystal X-ray analysis. Crystal data : Monoclinic, space group $P2_1$, a = 25.274 (4), b = 8.238 (3), c = 13.084 (3) A°, β = 91.56 (6)° and Z = 4. 4377 (2396 > 2σ) independent reflections were scanned on a four-circle automatic diffractometer using graphite-monochromatized Cu-Kα radiation. The structure was solved using the Patterson search method [4] with a selected starting set of coordinates from the marcfortine A ring system [1]. The initial rigid model contained 21 atoms. The best figure of merit was the starting point of Fourier recycling procedures which converge to R = 26 % and revealed the whole set of the 66 atoms of the two independant molecules of the asymetric unit. Isotropic refinement [5] was conducted to R = 13 % with hydrogen atoms introduced at their theoretical places. No anisotropic refinement was performed because of the paucity of the data.

The molecular structure of marcfortine C 3 is shown in the Figure. The ^1H n.m.r. and ^{13}C n.m.r. (Table I and II) spectra were consistent with

this structure. The electron impact mass spectrum of 3 displayed a fragment ion at m/z 404 (due to the loss of NHCO) and also showed abundant fragment ions in the medium mass range at m/z 177 and 149 (base peak) (see scheme 1).

The similar mass spectral fragmentation patterns of marcfortines B and C and the virtual identical resonances from 0.81 to 3.72 ppm in their ^{1}H n.m.r. spectra (Table I) strongly supported the proposed structure 2 for marcfortine B.

Marcfortines A, B and C seem to be biogenetically derived from two units of dimethylallyl-pyrophosphate, pipecolic acid and tryptophan. The loss of the carbonyl oxygen of tryptophan in the supposed formed dioxopiperazine ring has been found previously in fungal alkaloids [6]. The formation of the six membered ring on the tryptophan unit in marcfortine C probably involves, as in the seven membered rings of marcfortine A and B, an epoxy C-6 reverse O-isopentenyl intermediate which after transpostion to the corresponding aldehyde undergoes intramolecular nucleophilic attack by the aromatic ring.

Quite recently [7], the structure of paraherquamide from Penicillium paraherquei has been reported. It also has a seven membered ring located on the tryptophan unit and differs from marcfortine A 1 [1] by the nature of the second aminoacid (2-hydro-2-methyl proline instead of pipecolic acid).

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REFERENCES AND FOOTNOTES

1. J. Polonsky, M.-A. Merrien, T. Prangé, Cl. Pascard and S. Moreau, J.C.S. Chem.Comm., 601 (1980).
2. P.M. Scott, M.-A. Merrien, and J. Polonsky, Experientia, 32, 140 (1976) ; J. Polonsky, M.-A. Merrien and P.M. Scott, Ann.Nutr.Aliment, 31, 693 (1977) ; P.M. Scott and B.P.C. Kennedy, J.Agric.Food Chem., 24, 865 (1976) ; P.M. Scott, B.P.C. Kennedy, J. Harwig, and B.J. Blanchfield, Appl & Envir. Microb., 33, 249 (1977) ; P.M. Scott, J. Polonsky, and M.-A. Merrien, J. Agric. Food Chem., 27, 201 (1979).
3. The spectra of 1 and 2 have been recorded on a Bruker HX 90 at 22.63 MHz, in $CDCl_3$ (δ in ppm downfield from TMS). The reported assignments [1] for C-16, C-15 and for some of the Me groups of 1 have been revised. The spectrum of 3 has been recorded on a Bruker WP 60 at 15.08 MHz using a microprobe, in $CDCl_3$ (δ in ppm downfield from HMDS (δ HMDS = 1.7 ppm).
4. B.P. Braun, J. Hornstra and J.L. Leenhouts, Philips Research Rapport, 24, 85 (1969).
5. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW.
6. R.F. Bond, J.C.A. Boeyens, C.V. Holzapfel and P.S. Steyn, J.Chem.Soc. Perkin Trans I, 1751 (1979).
7. M. Yamazaki, E. Okuyama, M. Kobayashi and H. Inoue, Tetrahedron Letters, 32, 135 (1981).

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