Isolation and Structure (*X*-Ray Analysis) of Marcfortine A, a New Alkaloid from *Penicillium roqueforti*

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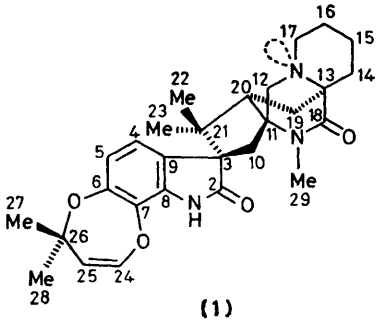
Summary The structure of marcfortine A, a novel alkaloid isolated from *Penicillium roqueforti*, has been established by *X*-ray analysis; two minor alkaloids, marcfortine B and C, as well as the previously known roquefortine have also been isolated.

Penicillium roqueforti is the essential fungus used in the production of the many varieties of blue cheese containing internal mould. Previous studies of the *P. roqueforti* strain CS1 resulted in the isolation and structural determination of two alkaloids: roquefortine[1-3] and isofumigaclavine A.[1,2,4] Investigation of the mycelium of the strain B26 of *P. roqueforti*† has now led to the isolation, along with roquefortine, of three new alkaloids which have been designated marcfortine A, B, and C. We herein report the structural elucidation of the major alkaloid, marcfortine A (**1**).



(1)

Chromatography of the alkaloidal extract (380 mg) obtained from lyophilized mycelium (196 g) of *P. roqueforti* (strain B26) gave roquefortine (24 mg), marcfortine A (**1**) (79 mg), and the marcfortines B and C.

The molecular formula of (**1**) ($C_{28}H_{35}N_3O_4$) was established by microanalysis and by mass spectrometry with $M^{+\cdot}$ at *m/e* 477·2602 and a fragmentation ion (100%) at *m/e* 418·2248 [$C_{26}H_{30}N_2O_3$ ($M^{+\cdot}$ — MeNHCHO)]; m.p. 242—244 °C, $[\alpha]_D^{22}$ −5·3° (*c* 1·14, $CHCl_3$); i.r. ($CHCl_3$) ν_{max} 3400 (NH) and 1700 and 1630 cm^{-1} (CO); u.v. λ_{max} 226 (ϵ 30080) and 268 nm (sh. ϵ 4625).

The 250 MHz ^1H and the ^{13}C n.m.r. spectra revealed a certain amount of structural information. The former showed that (**1**) has an aromatic ring which has two contiguous protons (H-4 and H-5) giving rise to an AB quartet (doublets at δ 6·79 and 6·61, *J* 8·1 Hz) and it also displayed a widely separated olefinic AB quartet (d at δ 6·38 and d at 4·92, *J* 7·5 Hz), assigned to H-24 and H-25, respectively. It furthermore revealed signals due to four methyl groups [δ 0·84, 1, 13, and 1·45 (6H)] suggesting the presence of two isoprene units.

Of the three nitrogen atoms, two are involved in NH (δ 8·7, exchangeable with D_2O) groups and one in an N–CH_3 (δ 3·12) group. The ^{13}C n.m.r. spectrum (Table) substantiated these findings and also showed that (**1**) contained one oxygen-bearing quaternary carbon (δ 79·8) and two C=O groups at δ 173·9 and 185·5 p.p.m. The chemical shift of the first is that of an amide function whereas the second would correspond rather to that of an indolinone.

† This strain was isolated in 1977 by Professor J. Pelhate (Brest, France) and proved to be *P. roqueforti* in 1979 by the 'Central bureau voor Schimmelcultures de Baarn (Hollande)'.

TABLE. ^{13}C N.m.r. spectrum of marcfortine A (**1**) (at 22·6 MHz, in $CDCl_3$, δ in p.p.m. downfield from Me_4Si).

C-2	185·3	S	C-11	63·1	S	C-20	52·9	D
C-3	60·6	S	C-12	61·5	T	C-21	46·5	S
C-4	120·2	D	C-13	64·2	S	C-24	139·2	D
C-5	114·9[a]	D	C-14	31·7	T	C-25	117·2[a]	D
C-6	146·2	S	C-15	25·9	T	C-26	79·8	S
C-7	135·4[b]	S	C-16	31·7	T	N–Me	29·9	Q
C-8	132·8[b]	S	C-17	54·5	T	21-Me	20·7	Q
							25·8[c]	Q
C-9	124·9	S	C-18	173·9	S	26-Me	20·7	Q
C-10	37·0	T	C-19	31·7	T		26·4[c]	Q

[a–c] Signals may be reversed.

Marcfortine A (**1**) was unaffected by acetylation (Ac_2O–pyridine) and methylation (EtOH–CH_2N_2) conditions.

All the foregoing results strongly indicated that (**1**) has the structure of a monoketopiperazine with two isoprenic units and a substituted indolinone system. For a complete structural analysis, however, a single crystal *X*-ray analysis was carried out using crystals of (**1**) obtained from ethyl acetate solution.

Crystal data: Orthorhombic, space group $P2_12_12_1$, $a = 6{\cdot}423$, $b = 15{\cdot}822(3)$, $c = 24{\cdot}226(5)$ Å, $Z = 4$. Intensity data for 2133 independent reflections were measured using Cu-K_α radiation. The structure was solved by direct methods using the multisolution technique,[5] and was anisotropically refined to a final conventional *R* factor of 6·3% ($R_w = 4{\cdot}7\%$) based on 1385 structural factors. (All H atoms were located by a difference Fourier synthesis.)

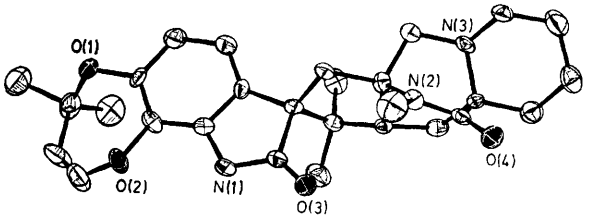
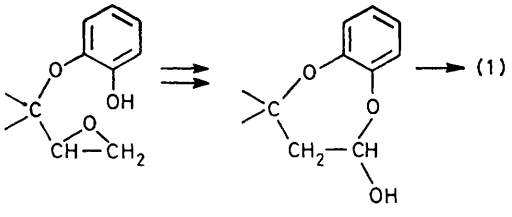


FIGURE. Molecular structure of marcfortine A (**1**).

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

Marcfortine A contains several interesting and new structural features. Biogenetically, the basic skeleton of marcfortine A is clearly derived from a dioxopiperazine formed from tryptophan and pipecolic acid. During this process the dioxopiperazine ring has been modified by loss of the carbonyl oxygen of tryptophan, as has been noted previously.[6] To our knowledge, (**1**) is the first fungal metabolite known to contain the pipecolic acid unit. Oxalin[7] and roquefortine are the only other alkaloids to have the reverse isoprene unit at C-3 of tryptophan which, in marcfortine A, is also linked to C-11 and C-13. The location of the second isoprene unit and the linkage to two phenolic hydroxy-groups on the tryptophan unit is a unique structural feature. The formation of this seven-membered ring can be explained by several mechanisms involving oxidative cyclisation of an isopentenyl ether. A plausible alternative is shown in equation (1).[8]



We are grateful to Mme. C. Fontaine and Mr. M. Vuilhorgne for the ^{13}C n.m.r. spectrum, Mr. C. Merienne (Laboratoire de R.M.N. à Haut Champs, Orsay) for ^1H n.m.r. measurements at 250 MHz, Rhône-Poulenc Co. (Centre Nicolas Grillet, Vitry) for the high resolution mass spectrum, and Mme. A. Masset for assistance with the cultures of *P. roqueforti*.

(*Received, 29th February* 1980; *Com.* 223.)

‡ 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. Any request should be accompanied by the full literature citation for this communication.

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[8] Mechanism suggested by Professor D. H. R. Barton.