The Structure of Polivione, a Polyketide Co-metabolite of Citromycetin in *Penicillium frequentans*

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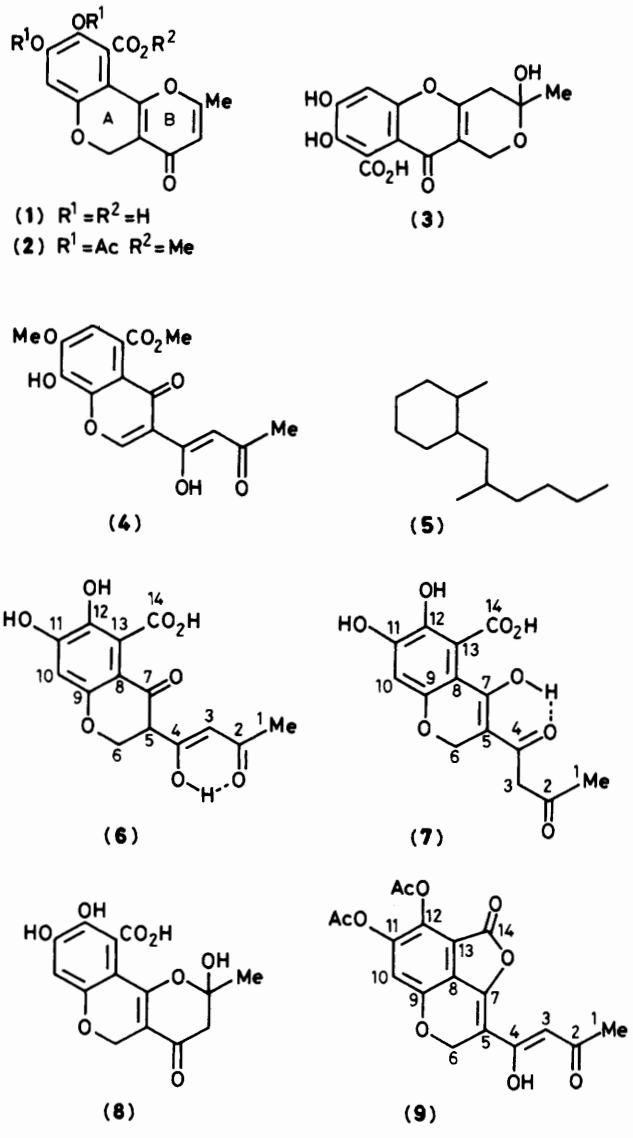
Polivione, a polyketide metabolite and probably a precursor of citromycetin, has been isolated from *Penicillium frequentans*

Citromycetin (**1**) is known to be polyketide derived, but the details of its biosynthesis remain obscure in spite of the many investigations of various groups. It was suggested, on the basis of early incorporation experiments using ^{14}C-labelled malonate, that two separately formed polyketide chains might be involved in the biosynthesis.[1] This view has been challenged in studies of the closely related polyketide fulvic acid (**3**), on the basis of experiments using ^{13}C-labelled acetate.[2] Another metabolite lapidosin (**4**), reported recently,[3] shares with (**1**) and (**3**) the same carbon skeleton (**5**) and is probably related in its biosynthesis. The interest of this biosynthetic family is also reflected in recent reports of synthetic approaches to (**1**) and (**3**) *via* supposedly biomimetic routes.[4]

As part of our continuing investigation of citromycetin biosynthesis, we have searched for possible biosynthetic intermediates. To our surprise, analysis of an ethyl acetate extract of a culture of *Penicillium frequentans*, by thin layer chromatography on SiO_2 (treated with dil. H_3PO_4 prior to drying), revealed that citromycetin is only a trace component of the mixture. The major component, which forms a green



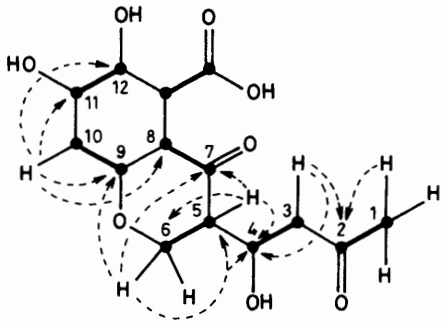


Figure 1. Sites of spin–spin couplings in ^{13}C n.m.r. spectra of polivione (**1**); heavy lines denote C_2-units showing strong ^{13}C–^{13}C coupling in polivione derived from [1,2-$^{13}C_2$]acetate; dotted arrows denote ^1H–^{13}C long range couplings detected by the 2D ^1H–^{13}C correlation experiment.

Table 1. ^1H N.m.r. spectroscopic[a] data of polivione, (**6**) and (**7**), and diacetylanhydropolivione, (**9**).

	Polivione tautomers		
Hydrogen	(**6**)	(**7**)	(**9**)[b]
1	2.05	2.3	2.15
3	5.5	3.6	6.26
5	3.65 (t, *J* 6 Hz)	—	—
6	4.53 (dd, *J* 6, 11 Hz)	4.8	5.46
	4.85 (dd, *J* 6, 11 Hz)		
10	6.7	6.65	6.82
OH	—[c]	—[c]	15.5

[a] At 250 MHz in $CDCl_3$ solution; all resonances singlets except where multiplets are specified. [b] Acetate resonances at δ 2.31 and 2.35. [c] Hydroxy resonances at δ 14.0, 14.5, and 15.0.

spot at higher R_f, was isolated by flash chromatography on acidified SiO_2, as an unstable yellow oil. We now present spectroscopic and chemical evidence that this new metabolite, which we name polivione, exists as a mixture of two slowly interconverting tautomers (**6**) and (**7**). We also show that it is readily converted into citromycetin (**1**) under conditions used in previous work for the isolation of that compound.

Table 2. Chemical shifts (δ) and couplings ($^1J_{CC}$) in the ^{13}C n.m.r. spectra of polivione, (**6**) and (**7**), and diacetylanhydropolivione, (**9**).

	Polivione tautomers[a]		
Carbon	(**6**)	(**7**)	(**9**)[b]
1	23.9 (46.2)	30.8 (42.6)	24.4 (46.0)
2	189.56 (46.1)	200.3 (42.5)	189.9 (46.1)
3	100.2 (62.6)	48.9 (49.2)	101.37 (61.9)
4	189.54 (62.5)	172.9 (49.2)	179.7 (61.9)
5	55.0 (34.4)	105.0 (49.5)	103.26 (45.1)
6	67.4 (34.4)	65.9 (49.5)	68.8 (45.2)
7	193.0 (58.1)	185.0 (58.1)	144.6 (52.7)
8	108.7 (58.1)	110.0 (58.0)	123.9 (52.7)
9	162.7 (69.7)	160.0 (69.8)	150.3 (74.0)
10	107.6 (69.6)	107.7 (69.9)	115.8 (74.0)
11	152.2 (64.2)	151.4 (65.4)	147.4 (84.7)
12	157.6 (64.2)	155.9 (65.3)	133.6 (84.4)
13	108.9 (65.8)	109.5 (65.7)	113.9 (79.4)
14	173.0 (65.9)	173.2 (66.2)	161.7 (79.3)

[a] At 100 MHz in CD_2Cl_2 solution (δ relative to CD_2Cl_2 = 53.85 p.p.m.). [b] At 100 MHz in $CDCl_3$ solution (δ relative to $CDCl_3$ = 77.02 p.p.m.); the four acetate resonances are at δ 20.3, 20.5, 167.2, and 167.6.

Polivione ($C_{14}H_{12}O_8$) had strong absorption bands in both the hydroxy and carbonyl regions of the i.r. spectrum ($\nu_{max.}$ 3500, 1726, and 1668 cm^{-1} in $CHCl_3$), consistent with a mixture of (**6**) and (**7**). The ^1H n.m.r. spectrum (Table 1) can be divided into sets of C–H peaks corresponding to the two isomers, the relative amounts of which can vary [from 1 : 1 to 4 : 1 in favour of (**6**) in $CDCl_3$ solution]; the assignments, made with the aid of homonuclear decoupling experiments, are consistent with intensity measurements. Addition of a drop of D_2O to a $CDCl_3$ solution resulted in immediate loss of strong OH signals at δ 14.0, 14.5, and 15.0; there was also a more gradual loss of those for H-3 and H-5, accompanied by expected changes elsewhere in the spectrum. The ^1H n.m.r. spectrum also showed evidence for a trace of a third tautomer to which we tentatively assign structure (**8**), on the basis of an AB quartet at δ 3.81 and 3.99 (*J* 16.5 Hz), assigned to the C-3 methylene hydrogens.

The ^{13}C n.m.r. spectrum (Table 2) of polivione was assigned by a combination of two dimensional correlation experiments, which allowed us to map the complete skeleton of C–C and C–H bonds for both tautomers, (**6**) and (**7**). Thus a carbon–carbon correlation spectrum[5] of polivione, enriched by biosynthesis from [1,2-$^{13}C_2$]acetate, showed ^{13}C–^{13}C couplings which established for tautomer (**6**) the carbon–carbon

connectivities indicated by heavy lines in Figure 1; the enrichment level was not sufficiently high for the detection of the ^{13}C–^{13}C couplings between adjacent C_2-units. The gaps could be bridged, however, by a combination of a standard ^{1}H–^{13}C two dimensional correlation experiment[6] to confirm the assignments of directly bonded nuclei, and a similar experiment optimised to detect equivalent long range couplings. The pattern of the latter is indicated by arrows in Figure 1. Of particular significance is the coupling between H-6 and C-9 which establishes the presence of the ether link. The rest of the functional groups fall logically and unambiguously into place, with the exception of the enolised β-diketone residue, C-2, C-3, and C-4. This could equally well be enolised in the opposite direction to that shown; indeed from the closeness of the chemical shifts of C-2 and C-4 we suspect that the two possible enol forms may be in rapid equilibrium on the n.m.r. time scale. Equivalent evidence was obtained to support (**7**) as the structure of the minor tautomer of polivione, again with the possibility that it may exist as two rapidly interconverting tautomers of the enolised β-diketone residue.

The chemical properties of polivione were investigated both to confirm its structure and to investigate its relationship to citromycetin (**1**). Firstly, it was converted into citromycetin in high yield when treated with HCl in aqueous methanol. This facile transformation confirms the structure, but it frustrated many attempts to prepare derivatives. Acetylation (Ac_2O–NaOAc), however, gave a crystalline anhydro derivative ($C_{18}H_{14}O_9$). This has n.m.r. spectroscopic properties (Tables 1 and 2) consistent with the proposed structure (**9**). The presence of an enol lactone was supported by the i.r. spectrum ($\nu_{max.}$ 1800 and 1790 cm^{-1} in $CHCl_3$). Chemical support for the structure came from its conversion into polivione (**6**) and (**7**) on treatment with aqueous acetic acid, and to the diacetyl citromycetin methyl ester (**2**) on treatment with HCl in aqueous methanol.

Our twin discoveries that polivione is the major component of extracts of *P. frequentans* and that it is readily converted chemically into citromycetin (**1**) raise doubts concerning the status of the latter compound as a natural product. Whatever the truth, it seems likely that polivione is the chemical or biochemical precursor of the small amount of citromycetin detected in crude extracts by our procedure, and therefore that ring A of (**1**) is formed before ring B. In a recent supposedly biomimetic synthesis of the citromycetin system the opposite strategy was employed.[4] This in no way detracts from the merits of the synthesis as such, but it does mean that the task of devising a close chemical model for the biosynthesis remains open. More detailed comment on the biosynthetic implications of our results is presented in the following paper.

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