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THE STRUCTURE OF ALBOFUNGIN

A.I.Gurevich, M.G.Karapetyan, M.N.Kolosov, V.N.Omelchenko, V.V.Onoprienko, G.I.Petrenko, S.A.Popravko

(Shemyakin Institute for Chemistry of Natural Products, USSR Academy of Sciences, Moscow, USSR)

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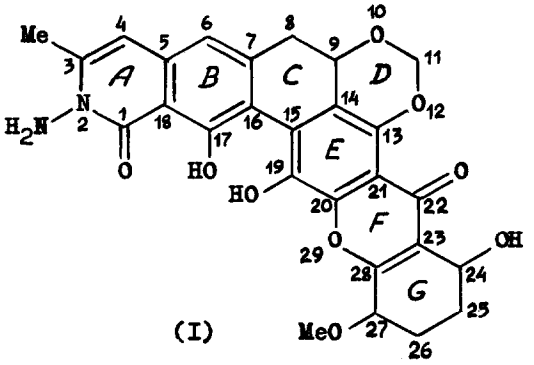
Albofungin is a metabolite of Actinomyces albus var. fungatus, highly active against Gram positive bacteria and yeasts (1,2). We have found it to be an antibiotic of a novel type structure I.



(I)

Albofungin, $C_{27}H_{24}N_2O_9$ (m.p.304-307°,from $MeNO_2$;$[\alpha]_D^{20}$-670°, in DMF; λ_{max}^{EtOH} 228, 254, 303, 364i, 376 nm (lg ε 4.58, 4.58, 4.19, 4.35, 4.42)) contains two conjugated C=O (ν^{KBr} 1652, 1623 cm^{-1}), an OMe on a saturated carbon and a (C)Me and two non coupled aromatic/heteroaromatic protons (cf. Fig.1). It yields a pentaacetyl derivative (m.p.230-233°) and exchanges for deuterium 5 hydrogens, accounted for by an NH_2 group (N-isopropylidene derivative: m.p.226-228°), two phenolic OH (dimethyl ether: m.p.298-300°) and a secondary alcoholic OH (δ^{DMSO} 5.07d, J 4.5).

Hydrolysis of albofungin by 0.5 N KOH in 50% dioxan at 100° affords in ca. 90% yield albofungol (II: m.p.294-295°), a degradation product $C_{21}H_{18}N_2O_7$ lacking the OMe and alcoholic OH of the parent antibiotic but retaining intact

its chromophore and moreover acquiring an extra phenolic OH and a chelated MeCO easily reducible by $NaBH_4$; NMR of the reduction product (17-monomethyl ether: m.p.290-292°, $\delta^{CDCl_3}_{CH(OH)Me}$ 5.68q, J 6.5) shows that the MeCO must be attached to an aromatic nucleus. Of the three hydroxyls of albofungol two are chelated and react readily with CH_2N_2 to give first a monomethyl ether (III: m.p.192-194°) and then a dimethyl ether (IV: m.p.157-160°). An isomeric dimethyl ether of II (V: m.p.174-175°) is formed on alkaline hydrolysis of albofungin dimethyl ether under the above conditions.

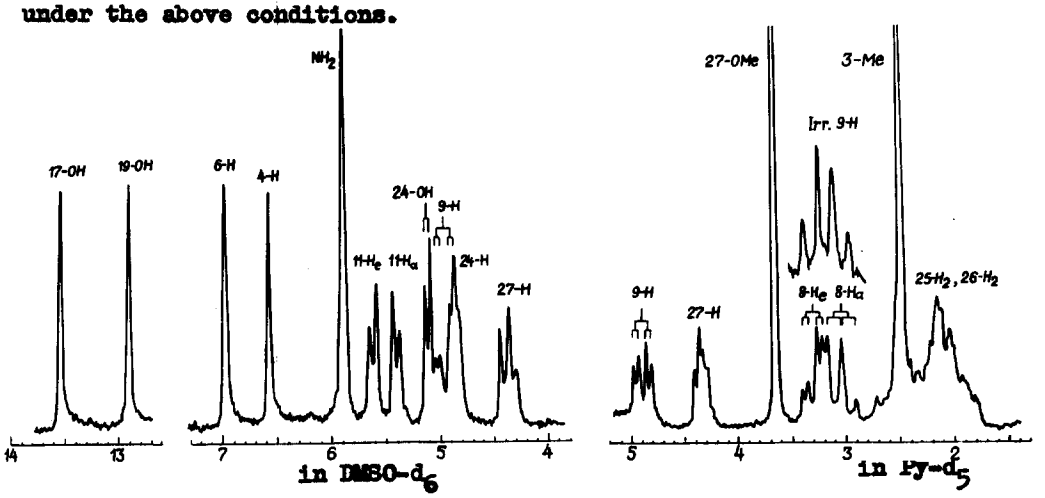
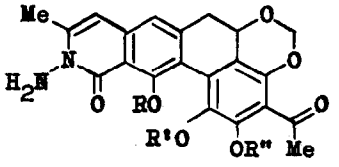


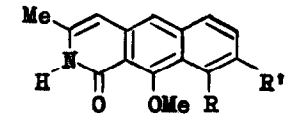
Fig.1. The NMR spectra of albofungin in DMSO-d_6 and Py-d_5 at 100 MHz.

Permanganate oxidation of the antibiotic and of III yielded 6-methyl-2(1H)-pyridone-3,4-dicarboxylic acid and methoxypyromellitic acid, respectively, from which followed that albofungin has an isoquinolone nucleus 6:7-condensed to another ring. The presence of the linear tricyclic system was corroborated by oxidation of III with alkaline 30% H_2O_2 into benzoisoquinolone acids IX - XI (IX, methyl ester: m.p.270-275°; X, methyl ester: m.p.185-187°; XI, γ-lactone methyl ester: m.p.218-220°). The structure of IX was proved by synthesis via ethyl 6-methyl-4(4'-cyanobenzyl)-2(1H)-pyridone-3-carboxylate; the Me-ester of X was oxidized by Me_2SO + Ac_2O to the corresponding keto ester which yielded IX on treatment with alkaline H_2O_2; the location of the extra carboxyl in XI was deduced from NMR data (retention of the pair of ortho-coupled protons: δ^{CDCl_3} 7.70d, 8.10d; J 8).

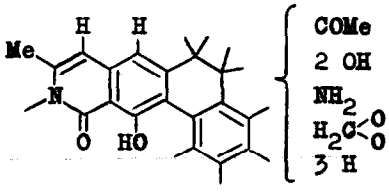
The acid XI lacks four of the twenty one carbons present in albofungol. Two of these were lost with the MeCO eliminated on oxidation of III. The third carbon must have closed the R and R' chains of XI into the benzene ring carrying the MeCO group. The fourth carbon was in a $-OCH_2O-$ group whose presence was revealed by formation of CH_2O on acid degradation (90% H_2SO_4, 100°) or on melting of albofungol. The data presented are summarized in XII.



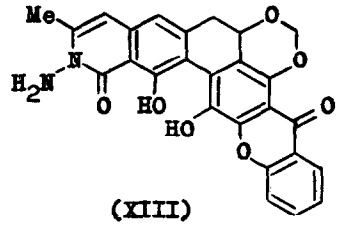
(II): R = H, R' = H, R'' = H
(III): R = Me, R' = H, R'' = H
(IV): R = Me, R' = H, R'' = Me
(V): R = Me, R' = Me, R'' = H
(VI): R = Me, R' = Me, R'' = Me
(VII): VI, H in place of NH_2
(VIII): VI, Me in place of NH_2



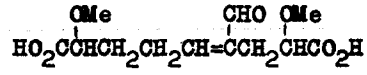
(IX): R = H, R' = CO_2H
(X): R = H, R' = $CH(OH)CO_2H$
(XI): R = CO_2H, R' = $CH(OH)CO_2H$



(XII)



(XIII)



(XIV)

The NMR (in d_5-Py) characteristics of the methylenedioxy group (δ 5.28d, 5.60d; J 6) and of the protons unidentified in the partial structure XII (ABX: δ_A 2.97, δ_B 3.23, δ_X 4.83; J_{AB} 13.5, J_{AX} 12.5, J_{BX} 5) are indicative of the fragment (Ar)-CH_2-CH-O-CH_2O-(Ar') the dioxa-ring of which should be six membered (cf. NMR of 4-benzyl-1,3-benzodioxan: $\delta_{CH_2}^{CDCl_3}$ 5.09d, 5.25d; J 6). Methylation of N-isopropylidene albofungol (m.p.269-270°) with MeI + K_2CO_3 followed by mild acid hydrolysis yielded trimethyl albofungol (VI: m.p.203-205°) which was reduced by Zn+AcOH to give the deamino compound (VII: m.p.161-163°) subsequently methylated to VIII (m.p.162-164°). One of the methyls present in VIII (and none in VII) was found to shift significally downfield on changing the solvent from $CDCl_3$ to CF_3CO_2H ($\Delta\delta$+0.55 ppm). Hence this methyl must be attached to

the pyridone nitrogen (cf. NMR of 2,3-dimethyl-8-methoxy-1(2H)-isoquinolone: $\Delta\delta_{N-Me}$ +0.55 ppm) indicating a hydrazide structure for albofungol, which accounts for elimination of the amino group on the Zn reduction.

The location of MeCO and two OH in the E ring was established as follows. In the ether IV the acetyl is non-chelated ($\nu_{C=O}^{KBr}$ 1710 cm^{-1}) being therefore remote from the only free hydroxyl. Methylation of the latter resulted in a marked upfield shift of the 17-methoxyl (IV→VI or III→V: $\Delta\delta_{17-OMe}^{CDCl_3}$ -0.2 ppm) showing proximity of these groups. Hence, the non-chelated hydroxyl and acetyl are in positions 19 and 21, respectively, and albofungol possesses the structure II.

Two products were isolated alongside II on the hydrolysis of albofungin. One of them (XIII: m.p.208-210°; yield 3%) was shown by NMR to lack the OMe and alcoholic OH of the antibiotic and to possess a o-disubstituted benzene ring. The second product proved to be the aldehydo acid XIV (yield 40%; 2,4-dinitrophenylhydrazone: m.p.185-187°) apparently resulting from self-condensation of the intermediate 2-methoxyglutaraldehydic acid. Isolation of XIII proved the presence in albofungin of a terminal cyclohexene ring (G) which includes C-23 (as a precursor of the Me(CO) of albofungol) and etherizes 20-OH of albofungol, thus forming a γ-pyrone cycle (F). The allylic position of the OMe and OH in this ring is evident from the NMR spectrum (Fig.1), while formation of the aldehydo acid XIV shows that the hydroxyl is β to 22-CO. There it follows the structure I for albofungin (3).

In Actinomyces albus spp. albofungin is accompanied by a closely related antibiotic $C_{27}H_{23}ClN_2O_9$ (m.p.327-330°, from $MeNO_2$; $[\alpha]_D^{20}$ -560°, in DMF) which was structurally elucidated as 4-chloro-I on the grounds of UV, IR, MS and NMR data.

REFERENCES

1. N.K.Solovieva, S.M.Rudaya, Antibiotiki, 4 (6), 5 (1959); A.S.Khokhlov, G.S. Rosenfeld, ibid., 4 (6), 10 (1959).

2. Albofungin is apparently identical with the antibiotic BA-180265 reported by W.-C.Liu, W.P.Cullen, K.V.Rao, Antimicrobial Agents & Chemotherapy-1962, 767.

3. The available data suggest a 24S,27R configuration for the antibiotic.