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ASCOFURANONE, A NEW ANTIBIOTIC FROM ASCOCHYTA VICIAE

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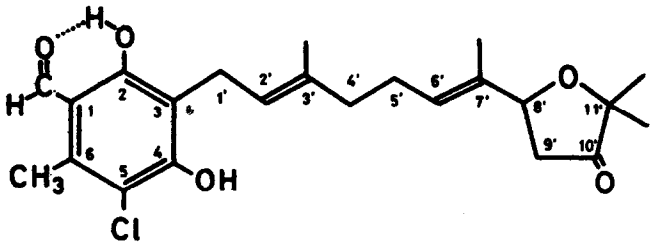
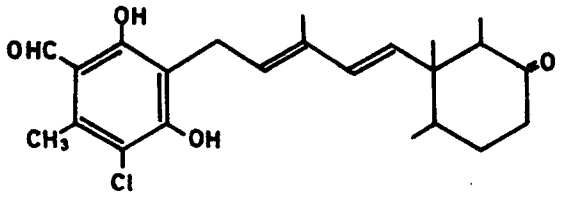
Isolation, biological properties and structural elucidation by X ray method of an antibiotic, ascochlorin (II), have been reported previously (1)(2)(3). The present paper deals with the structure of a new antibiotic, ascofuranone (I), which is structurally related to ascochlorin. Ascofuranone was isolated from the filter cake of the fermented broth of Ascochyta viciae Libert, a phytopathogenic fungus which produces ascochlorin. Ascofuranone shows positive Beilstein and $FeCl_3$ reactions. Two moles of bromine were consumed per mole of the antibiotic. Ascofuranone was recrystallized from n-hexane-acetone (2:1) as colorless fibrous needles, mp 84 °C, $[\alpha]_D^{25}$ -50 ° (c 1, in methanol), analysis; found C 65.24 %, H 6.93 %, Cl 8.26 %; calculated for $C_{23}H_{29}O_5Cl$, C 65.62 %, H 6.94 %, Cl 8.43 %. The Rf value was 0.42 on silica-gel



(I)



(II)

thin-layer plate by the solvent system of petr. ether-acetone (3:1), whereas that of ascochlorin was 0.35. The molecular formula, $C_{23}H_{29}O_5Cl$, was assigned on the basis of the elementary analyses and the molecular weight, 420, determined by mass spectroscopy.

The presence of the same 5-chloroorcylaldehyde moiety as ascochlorin was indicated in the structure of ascofuranone by the uv., ir., nmr., and mass spectra. The ir spectrum (KBr disc) showed intense bands at 3320 (OH), 2970-2860 (methyl, methylene and methine) and 1735, 1635 cm^{-1} (carbonyl). The spectrum was closely similar to that of ascochlorin except that the former exhibited a shifted carbonyl band from 1710 to 1735 cm^{-1} and absence of an intense band at 965 cm^{-1} which is due to conjugated trans double bonds in pentadiene chain of ascochlorin. An intramolecular H-bonding was indicated by a broad band centered at 2800 cm^{-1} and a strong carbonyl band at 1635 cm^{-1}. The uv. absorption spectrum exhibited three absorption maxima at λ_{max}^{EtOH} 228 nm (ε 20.300), λ_{max}^{EtOH} 295 nm (ε 15.000) and λ_{max}^{EtOH} 350nm (ε 8.200), suggesting that ascofuranone possesses 5-chloroorcylaldehyde moiety. Validity of this assumption was supported by the uv. spectra recorded in 0.1 N ethanolic HCl and 0.1 N ethanolic NaOH in which both the shifts in λ_{max}^{EtOH} and the changes in molar absorptivity were comparable to those of ascochlorin. All the signals due to 5-chloroorcylaldehyde moiety of ascochlorin were also present at the same δ values in the nmr spectrum of ascofuranone (60 MHz, solvent $CDCl_3$, TMS as internal standard); that is, an intramolecular H-bonding phenolic proton (δ 12.68), phenolic proton (δ 6.55), an aldehyde proton (δ 10.12) and methyl protons (δ 2.50). The base peak was observed at m/e 199 in the mass spectrum which accompanied a characteristic peak at m/e 201, indicating that the fragment contains a chlorine atom. In the previous study on ascochlorin, this peak was assigned to a tropilium ion derived from fission between C-1′ and C-2′. Thus, it is evident that ascofuranone consists of 5-chloroorcylaldehyde moiety and a side chain attached at C-3 of the phenyl group.

The structure of the side chain was determined as follows. First, a terpenoidal nature was suggested by the side chain composition, $C_{15}H_{23}O_2$, and the presence of 4 additional methyl groups in the nmr. spectrum. The signal of methylene attached at C-3′ (δ 3.38, d, J=7cps) shifted to slightly higher field (0.1 ppm) than that of ascochlorin and coupled with olefinic proton at δ 5.21. This fact suggested that ascofuranone possesses a partial structure of phenyl-ring-CH_2-CH=C(CH_3)-CH_2-CH_2- instead of 3-methylpenta-2,4-diene in ascochlorin (4). Coupling between the methylene protons at C-1′ (δ 3.38) and the olefinic proton at C-2′ (δ 5.21) was confirmed by spin-spin decoupling. Lack of a strong band at 965 cm^{-1} was consistent

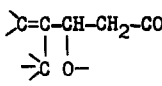
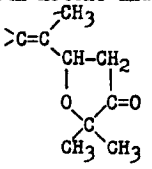
with the partial structure. Signals at δ 12.68 and 6.55 disappeared by adding D_2O, indicating that the former is an intramolecular H-bonded phenolic proton and the latter a phenolic proton, respectively. On irradiation at either δ 4.50 or 2.39, the other signal among the pair collapsed into a singlet. Unusually low chemical shift of the methine proton at δ 4.50 led to the following partial structure;  . On $NaBH_4$ reduction of the antibiotic, the product no longer showed carbonyl band, supporting that the carbonyl band at 1735 cm^{-1} ascribes to ketone, probably 5-membered cyclic ketone. Two methyl groups at δ 1.20 and 1.26 were aliphatic methyls which attached to the carbon atom adjacent to both ketone and etherial oxygen. These evidences showed the presence of the partial structure:  Two singlets at δ 1.63 and 1.79 are methyl groups attached to olefinic double bonds. Adjacent two methylene groups were present at δ 2.07 (m, 4H) between two olefinic double bonds in the heptadiene chain. The nmr. signals and their assignments are listed in the table.

Table. Nmr. spectrum of ascofuranone (solvent $CDCl_3$, TMS as an internal standard)

Chemical shift (δ)	Number of proton	Shape*	Assignment
12.68	1	s	Intramolecular H-bonded phenolic proton
10.12	1	s	Aldehyde proton
6.55	1	s	Phenolic proton
5.50	1	m	Olefinic proton at C-5′
5.21	1	t	Olefinic proton at C-2′, J=7 cps
4.50	1	t	Methine proton at C-8′, J=8 cps
3.38	2	d	Methylene at C-1, J=7 cps
2.50	3	s	Methyl attached to phenyl
2.39	2	d	Methylene at C-9′, J=8 cps
2.07	4	m	Two methylene at C-4′ and 5′
1.79	3	s	Methyl attached to C-7′
1.63	3	s	Methyl attached to C-3′
1.20 & 1.26	6	2 s	Methyl attached to C-11′

Thus, on the basis of these evidences, we propose the structure of ascofuranone as I. The structure is consistent with isoprene rule and readily interpretable the fragmentation pattern of the mass spectrum.

Ascofuranone differentiates from ascochlorin in the sesquiterpenyl side chain and their biogenesis are much interesting. The studies on the biogenesis as well as absolute configuration at C-8′ in ascofuranone are now under way. Although the antibiotic shows no antimicrobial activity, it is effective against some viruses in the agar-diffusion plaque-inhibition method. In addition, the antibiotic exerts serum lipid lowering property in mice when orally administered. Biological properties and details of this study will be presented elsewhere.

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* The following abbreviations are used ; s singlet, d doublet, t triplet and m multiplet.