ISOLATION AND STRUCTURE OF ASCOFURANONE AND ASCOFRANOL, ANTIBIOTICS WITH HYPOLIPIDEMIC ACTIVITY

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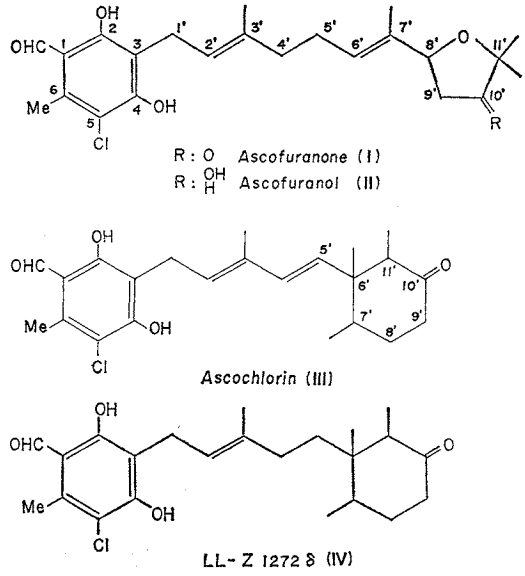
A new antibiotic with hypolipidemic activity, ascofuranone, $C_{23}H_{29}ClO_5$, and related substance, ascofuranol, $C_{23}H_{31}ClO_5$, were isolated from the filter cake of the fermented broth of *Ascochyta viciae* LIBERT, an ascochlorin-producing fungus, and their structures were elucidated. They possess 3-substituted-5-chloro-orcylaldehyde moiety with novel sesquiterpenyl side chains.

In our screening for hypolipidemic agents from microorganisms, new prenylphenols[1], ascofuranone[2] (I) and ascofuranol (II), were isolated from an ascochlorin-producing fungus, *Ascochyta viciae* LIBERT[3,4]. In this paper the isolation and structure of these novel prenylphenols are described.

Mutants of *A. viciae* were isolated by nitrosoguanidine treatment to improve ascochlorin yield. Two new prenylphenols were found in the mycelial acetone extract of a mutant No. 34, when the extract was subjected to silica gel thin-layer chromatography using *n*-hexane - acetone (4: 1) as a developing solvent. The mutant was grown in 30-liter jar fermentor containing the medium previously reported[3]. After one week of submerged fermentation, the mycelium was collected by filtration and extracted with a mixture of chloroform - methanol (9: 1). The chloroform extract was evaporated *in vacuo* and the residue soaked in *n*-hexane. Ascochlorin (III) crystallized slowly in a refrigerator and was removed by filtration. The mother liquor was again evaporated and the residue chromatographed on a column of silica gel. Elution with *n*-hexane - acetone (95: 5) yielded ascofuranone (I), $C_{23}H_{29}ClO_5$, mp 84~85°C, $[\alpha]_D^{21}$ −50° (*c* 1, methanol); further elution with *n*-hexane - acetone (4: 1) yielded ascofuranol (II), $C_{23}H_{31}ClO_5$, mp 75°C, $[\alpha]_D^{21}$ −7° (*c* 1, methanol), Rf values on Merck silica gel G plate, 0.42 for I, 0.23 for II and 0.35 for III.

Fig. 1. Structures of ascofuranone and related substances.

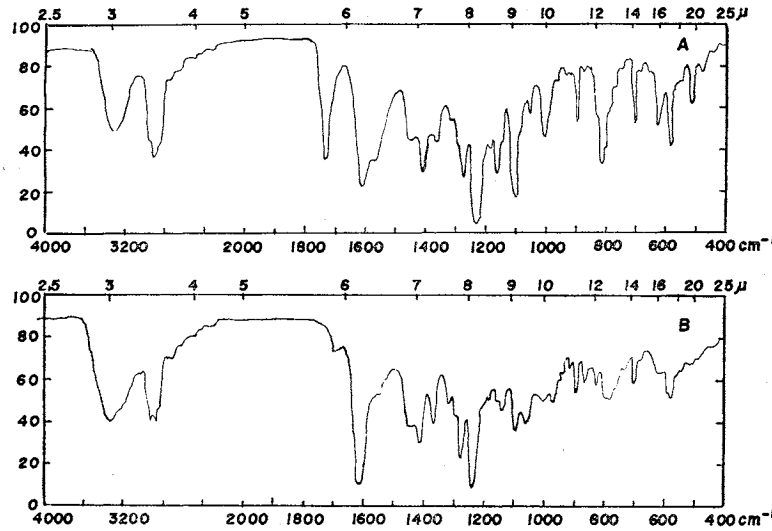


I and II are colorless needles from *n*-hexane - acetone (4: 1) and showed positive BEILSTEIN and ferric chloride tests. The presence of two olefinic bonds was evident from consumption of two moles of bromine per mole, and a tetrahydro-derivative was formed by catalytic hydrogenation.

Table 1. UV absorption maxima

	max (molar absorptivity)			Shift of principal ET band at alkaline condition (nm)
	LE band (nm)	ET band I (nm)	ET band II (nm)	
Ascochlorin	250 (29,600)	290 (10,600)	350 (11,000)	56
Ascofuranone	228 (20,300)	290 (15,000)	350 (8,200)	57
Ascofuranol	228 (15,800)	290 (8,700)	352 (13,900)	57
LL-Z1272	228 (16,400)	292 (9,400)	356 (13,500)	56

Fig. 2. IR spectra of ascofuranone (A) and ascofuranol (B)



The UV absorption spectra of **I** and **II** (Table 1) were strongly reminiscent of the 3- substituted-5-chloroorcylaldehyde system[5] as were the mass (Figs. 3, 4), NMR (Table 2), and IR (Fig. 2) spectral properties. The three absorption maxima with high molar absorptivity are characteristic for a phenol with a chelated carbonyl such as *o*-hydroxyacetophenone and **III** (Table 1). The principal ET bands of **I**, **II** and **III** are nearly the same, indicating that **I** and **II** posesses the same chromophore as **III**. This was further supported by the bathochromic shift, 57 nm, at basic condition. The UV spectra of **I** and **II** are practically indistinguishable from that of an ascochlorin analog, LL-Z1272 δ (**IV**)[6]. This fact means that **I** and **II** differentiate from **III** in unconjugated side chains like **IV**[5]. The partial structure is corroborated for the following reasons: (A) a common base peak, *m/e* 199, which is a tropilium ion[7] derived from side chain fission between C-1′ and C-2′, appears in all mass spectra (MS); (B) lack of the IR absorption band at 965 cm^{-1} which is assignable to a transconjugated diene in **III**; (C) the methylene attached at C-3 appears 0.13 ppm higher than that of **III**; (D) the signal of the methyl attached at δ 2.56, indicating that the methyl is shifted to low field by an adjacent chlorine; (E) a strong carbonyl absorption around 1630 cm^{-1} is in accord with 2,4-dihydroxybenzaldehyde system[8].

An isoprenoidal origin of the side chain was suggested by the composition, $C_{15}H_{23}O_2$, and the four methyls at δ 1.20, 1.26, 1.63 and 1.79 of **I**; the former two are aliphatic geminal dimethyls and the latter two those attached to olefinic bonds. On irradiation of a doublet at δ 3.38 (a methylene attached at C-3), a triplet at δ 5.21 (an olefinic proton at C-2′) collapsed into a singlet. This isolated

coupling system allowed us to assign them as aromatic ring $-CH_2-CH=\underset{\underset{CH_3}{|}}{C}-C-$. The chemical shift and intensity of the signal at δ 2.07 clearly showed the presence of adjacent methylenes inserted between olefinic bonds. Thus, the partial structure is expressed as follows: aromatic ring $-CH_2-CH=\underset{\underset{CH_3}{|}}{C}-CH_2-CH_2-\underset{|}{C}=C<$. On irradiation of a triplet at δ 4.50 (1H), a methylene doublet at δ 2.39 (adjacent to C=O) collapsed into a singlet and the unusually low chemical shift of a methine at δ 4.50, in addition to the isolated coupling system, led to the following partial structure; $>C=\underset{|}{C}-\underset{\underset{CH_3}{|}}{CH}-CH_2-C=O$. On $NaBH_4$ reduction of I, dihydroxy derivative was formed, which no longer showed a carbonyl band in the IR absorption spectrum. Therefore, a remaining oxygen functionality should be etherial, since the presence of β-lactone or alcohol was excluded. The chemical shift of the gem-dimethyl at δ 1.20 and 1.26 is consistent with β-position from both ketone and etherial oxygen. The evidences provided above established the structure of **I** as shown in Fig. 1. The MS data (Fig. 3) are in full agreement with the structure.

Table 2. NMR Spectrum of ascofuranone and ascofuranol

Functionality	Chemical shift (δ) and shape*	
	Ascofuranone	Ascofuranol
C-1—Aldehyde	10.12 s	10.11 s
C-2—Chelated OH	12.68 s	12.66 s
C-4—OH	6.55 s	6.98 s
C-6—Methyl	2.50 s	2.58 s
C-1′	3.38 d	3.37 s
C-2′	5.21 t	5.18 t
C-3′—Methyl	1.63 s	1.60 s
C-4′, 5′	2.07 s	2.06 s
C-6′	5.50 m	5.48 m
C-7′—Methyl	1.79 s	1.78 s
C-8′	4.50 t	4.30 t
C-9′	2.39 d	2.35 d
C-11′—Dimethyl	1.20 & 1.26 2s	1.20 & 1.26 2s
C-10′—OH		2.38 s
C-10′—H		3.95 t

Solvent $CDCl_3$, TMS as an internal standard
* The following abbreviations are used; s singlet, d doublet, t triplet, and m multiplet.

The IR spectral characteristics of **II** are very similar to those of **I**, but differ in lack of a carbonyl band at 1735 cm^{-1}. In contrast, O–H stretching vibration centered at 3300 cm^{-1} deepened and widened, and a new alcoholic stretching vibration appeared at 1090 cm^{-1} (secondary alcohol). When the NMR spectrum was compared with that of **I**, a new triplet (1 H) appeared at δ 3.95 accompanying with a new alcoholic signal at δ 2.39. The new triplet is clearly assignable to a methine with a hydroxyl.

Fig. 3. Fragmentation pattern of ascofuranone.

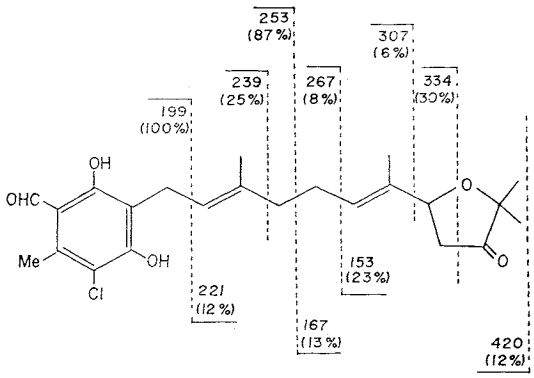
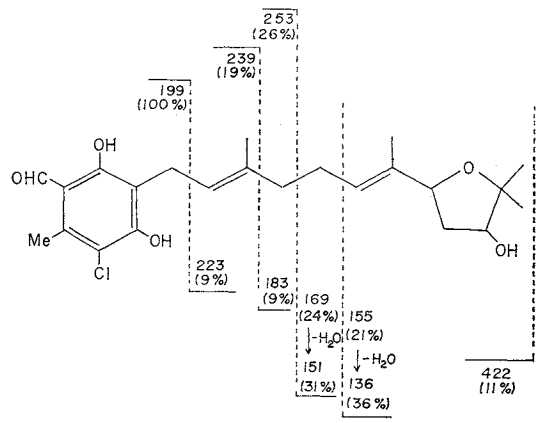


Fig. 4. Fragmentation pattern of ascofuranol.



Instead of the doublet at δ 2.39 of **I** a new multiplet appeared around δ 2.00 and a methine proton at C-8′ shifted 0.2 ppm higher than that of **I**. The evidence provided above is consistent with the structure of **II** as shown in Fig. 1. In fact, **I** could be obtained from **II** by oxidation with chromium trioxide-pyridine complex[9], although the yield was as low as 20%. The MS data of **II** afforded conclusive support. As shown in Fig. 4, all the fragment ions generated by fission between C-1′–C-2′, 3′–4′, 5′–6′ and 7′–8′ were observed in the mass spectrum.

Since the isolation of ascochlorin in 1968, the class ascochlorin containing 5-chloroorcylaldehyde with 3-substituted terpenyl side chain have been isolated from many fungi such as *Ascochyta viciae*, *Fusarium* species[6], *Colletotrichum*[10,11], *Cylindrocladium ilicicola*[12], *Cylindrocladium* species[13] and *Nectaria*[14]. However, **I** and **II** are the first in view of the novel sesquiterpenyl side chains of hydrofuranone structure. Stereochemistry of **I** and **II** remaining is now under way using X-ray method.

I is less toxic to mice and rats than **III** and effectively lowers serum lipid levels. The hypolipidemic property of **I** will be reported in a subsequent paper[15].

Experimental

Melting points were taken on a micro hot-stage apparatus and are uncorrected. IR absorption spectra were determined as KBr disk, and UV spectra for solutions in methanol. NMR spectra were obtained at 60 and 100 MHz in $CDCl_3$ with tetramethylsilane as an internal standard. Molecular weights were taken from the parent peaks in the low resolution mass spectra. Merck silica gel G was used in thin-layer chromatography.

Isolation of ascofuranone and ascofuranol: The *Ascochyta viciae* mutant strain No. 34 was grown in 30-liter jar fermentors for 7 days at 27°C. The fermented broth was harvested and the chromatographic separation of **I** and **II** was carried out as previously described[3]. Ascofuranone; found C 65.24, H 6.93, Cl 8.26%: M^+ 420; $C_{23}H_{29}ClO_5$ requires C 65.62, H 6.94, Cl 8.43%; M^+ 420. It is insoluble in water, slightly soluble in *n*-hexane, readily soluble in most organic solvents. It gave an intense purple-brown color with ferric chloride in ethanol. Ascofuranol; found C 65.31, H 7.35, Cl 8.42%; M^+ 422. The solubility was nearly the same as that of **I**. Thin-layers of silica gel impregnated with **I** and **II** become green on sprying with sulfuric acid at 110°C for 5 minutes.

$NaBH_4$ reduction of **I**: **I** (50 mg) was dissolved in 5 ml methanol on cooling in an ice-bath. $NaBH_4$ was added to the solution and the reaction mixture was stirred for 1 hour in an ice-bath. Then, excess $NaBH_4$ was decomposed by adding 10% aqueous acetic acid. The mixture was concentrated *in vacuo* to syrup. Water (10 ml) was added to the syrup and resultant emulsion was extracted with ethylacetate (10 ml × 2). After dehydration of the combined extract with Na_2SO_4, the extract was concentrated *in vacuo* to dryness. The product was a colorless oil; found C 65.12, H 7.81, Cl 8.28%; $C_{23}H_{33}ClO_5$ requires C 65.02, H 7.78, Cl 8.37%; M^+ 424, MW 424.5, ν_{max}^{film} 3400, 2970, 2903, 2850, 1420, 1228, 1100 cm^{-1}.

CrO_3 oxidation of **II**: **II** (423 mg) was dissolved in dry dichloromethane (10 ml) and was added in one portion to the magnetic stirred oxidizing solution (5% solution of chromic trioxide-pyridine complex in dry dichloromethane, 31 ml). After being stirred at room temperature for 15 minutes, the reaction mixture was filtered and the solvent was removed leaving the oily product (407 mg) which was purified by the preparative thin-layer chromatography with *n*-hexane- acetone (4: 1); yield of **I** was 83 mg (19.7%).

Catalytic hydrogenation of **I**: A solution of **I** (84 mg) in 10 ml of cyclohexane - ethanol (9: 1), was stirred overnight with 10 mg of 10% Pd-charcoal under an atmosphere of H_2, during which time approximately 2 moles of H_2 was adsorbed. After filtration to remove the catalyst and evaporation of the solvent, the crude reduction product was subjected to preparative thin-layer chromatography on 30 g silica gel plate, was extracted with acetone and concentrated to give colorless oil (52 mg). Found C 65.32, H 7.92, Cl 8.28%; $C_{23}H_{33}ClO_5$ requires C 65.02, H 7.78, Cl 8.37%. M^+ 424, MW 424.5.

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