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STRUCTURE OF C_{16}-TERPENES FROM ACROSTALAGMUS NRRL-3481

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The lactone (I) obtained from the culture of Acrostalagmus NRRL-3481[1] has been found to have a strong inhibitory activity on the growth of an Avena coleoptile section[2,3]. A previous report from these laboratories described that this C_{16}-terpenoid lactone (I) is biosynthesized from a diterpenoid precursor with loss of four carbon atoms[2]. In the course of this biosynthetic investigation, we find that strain of Acrostalagmus NRRL-3481 produces three new C_{16}-terpenoids (IV, VIII, X), some of which are assumed to be biosynthetic intermediates for the lactone (I).

Acrostalidic acid, mp 210-211°, M^+ 278.150 (calcd. 278.152), $C_{16}H_{22}O_4$, shows ir absorptions at 3300-2600, 3030, 1740, 1690cm^{-1} and nmr signals as shown in Fig.1. These spectral properties and the co-occurence of this metabolite with (I) in the culture suggested the structure (IV) for the acid. The high field methyl signal at δ 0.75ppm is assigned to the C-10 methyl group situated on cis 1,3-diaxial relationship with the C-4 carboxyl group. The stereochemistry of B/C ring junctions is assigned as trans from the two diaxial coupling constants, J_{8-14}=11.5 and J_{9-11}=12.5Hz. This structure is confirmed by decoupling experiment on the nmr. Irradiation of H_8 brings signal changes of H_6 and H_7 from ddd to dd, together with from dd of $H_{14\alpha}$ and $H_{14\beta}$ to d. Two allyl coupling constants, J_{5-7}=3 and J_{6-8}=2.5, and a very large homoallyl coupling constant, J_{5-8}=4.5, reveal that the two protons, H_5 and H_8, are oriented

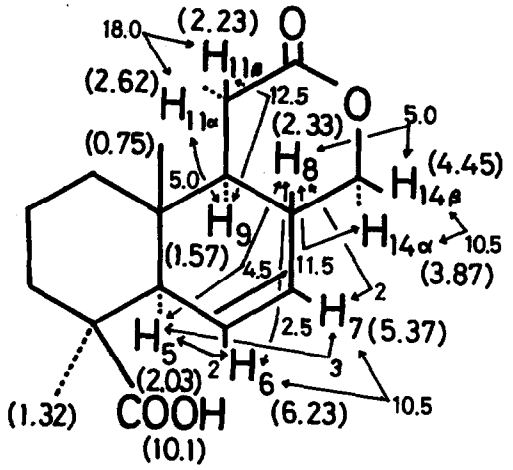
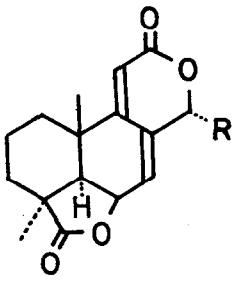


Fig. 1. Chemical shifts(in parenthesis) and coupling constants in the nmr spectrum of acrostalidic acid.

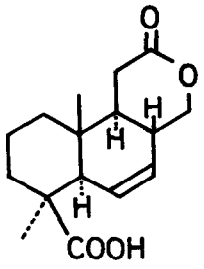
perpendicular to the plane of an ethylene bond. This relationship shows trans A/B ring junctions. As a corroboration of the skeleton of the acid (IV), ms fragmentations of dihydro acid (V) are very similar to those of (VI), which is obtained from a lactone (III) by catalytic hydrogenation. The lactone (III), prepared from lactol (II) by sodium borohydride reduction, is found to inhibit remarkablly the plant growth at the concentaration of 1ppm[6].

Acrostalic acid, mp 219-220°, M^+ 280.169 (calcd. 280.167), $C_{16}H_{24}O_4$, shows ir absorption at 3300-2600, 1700, 1690, 890cm^{-1}, and nmr peaks at 0.66(3H,s), 1.23(3H,s) 4.55(1H,br.s), 4.74(1H,br.s). These spectral properties suggest the structure (VIII) for this acid. The ms spectrum supported the structure. In agreement with the fragmentation patterns[7] of bicyclic diterpenes with C-4 carboxyl and C-8 terminal methylene groups, peaks at m/e 167(60%), 139(33%), and 121(100%) were observed in ms of (VIII). Direct comparisons of the dimethyl ester (IX) with the authentic compound[4], which had been synthesized from podocarpic acid, confirmed the structure including the absolute stereochemistry.

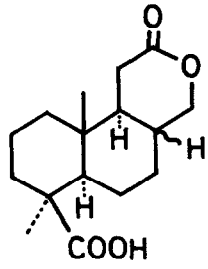
Isoacrostalidic acid, mp 204-206°, M^+ 278.152 (calcd. 258.152), $C_{16}H_{22}O_4$, ir 3300-2600, 3050, 1745, 1690cm^{-1}, δ 6.45(1H,dd,J=10.5, 2), 5.85(1H,dd,J=10.5, 3), 1.40, 1.34, 0.73(each s, 3H), has been deduced to have the structure (X) from the spectral similarities to acrostalidic acid(IV). The major differences are seen in ir and nmr; the isomer has a γ-lactone band at 1745cm^{-1} and a



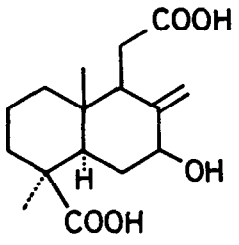
(I) R=CH_3
(II) R=OH
(III) R=H



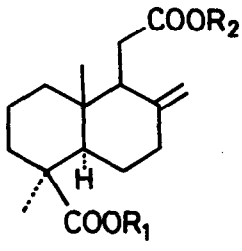
(IV)



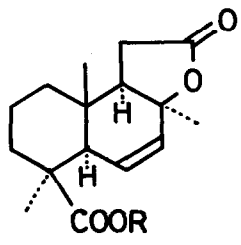
(V) β-H at C-8
(VI) α-H at C-8



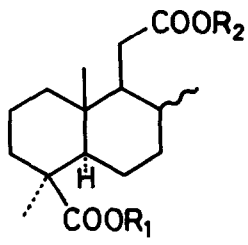
(VII)



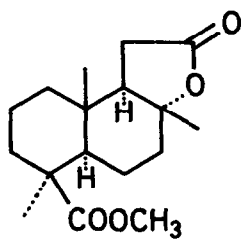
(VIII) R_1=R_2=H
(IX) R_1=R_2=CH_3



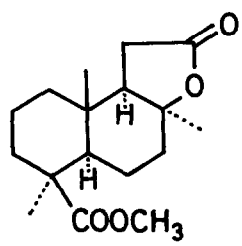
(X) R=H
(XI) R=CH_3



(XII) R_1=R_2=H
(XIII) R_1=R_2=CH_3
(XIV) R_1=CH_3, R_2=H



(XV)



(XVI)

methyl singlet at δ 1.40ppm instead of δ-lactone band at 1740cm^{-1} and oxomethyl signals around at δ 4.0ppm of acrostalidic acid. Catalytic hydrogenation of the lactonic ester (XI) afforded a saturated ester-acid (XIV) with hydrogenolytic cleavage of lactone ring, which dictates the positional correlation between γ-lactone group and ethylenic double bond. The carbon skeleton and stereochemistry around the A/B rings are confirmed from the fact that catalytic hydrogenation of the lactone (III) followed by methylation with diazomethane and partial hydrolysis with potassium hydroxide afforded the same ester-acid (XIV) that obtained from isoacrostalidic acid. The stereochemistry of the juctions of B-ring/lactone-group was assigned as cis from ir absorption 1745cm^{-1}. On brief acid treatment, monomethyl ester of acrostalic acid (VIII, R_1=CH_3, R_2=H) showed transiently an ir absorption band at 1770cm^{-1}, which showed a formation of trans lactone (XV). This trans lactone was readily changed to cis lactone (XVI), which showed a γ-lactone absorption at 1745cm^{-1} in accordance with the ir absorption of isoacrostalidic acid.

From the structures of these metabolites and the biosynthetic studies[2], a pathway for biosynthesis of the lactone (I) is pressumed as follows: a diterpenoid precursor such as labdadienol ⟶ VIII ⟶ VII $\xrightarrow{5)}$ (X) ⟶ IV ⟶ II ⟶ I.

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6) The biological activity test was carried out by courtesy of Dr. Y.Hayashi at the Osaka City University, to whom we are grateful.

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