Isolation and Identification of Tubaic Acid and β -Tubaic Acid from Derris Roots

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In the course of our screening research for biological active substances of *Derris elliptica* Benth., Leguminosae, we have isolated tubaic acid (I) and β -tubaic acid (II) as antimicrobial constituents from the roots. In this paper we wish to report the isolation and identification of the compounds.

Purification of the active constituents was followed by an assay of the growth inhibition of *Bacillus subtilis* according to the pulp disc method. Air-dried commercial derris roots were ground and extracted with boiling ethanol. The extract was fractionated into strongly acidic, weakly acidic and the other portions in the usual way. The strongly acidic fraction, showed an antimicrobial activity, was chromatographed on a silica gel column using *n*-hexane-ethyl acetate as an eluent. A fraction of *n*-hexane containing 5% ethyl acetate gave crude active compounds, which were further purified by repeated silica gel column chromatography to afford two antimicrobial constituents, I and II. The yields of I and II were 0.01% and 0.005% of air-dried roots, respectively.

I R=H

Ia R=CH₃

II R=H

IIa R=CH₃

The compound I, colorless needles from *n*-hexane: mp 128~129°C, $[\alpha]_D^{25} -73.0$ ($c=1.0$, MeOH), was positive to 2, 4-dinitrophenylhydrazine and ferric chloride tests. The spectral data of I were as follows: MS m/e : 220 (M^+), 202 (M^+-H_2O), 187, 176, 161; UV λ_{max}^{EtOH} nm (ϵ): 214 (27000), 222 shoulder (19400), 264 (11200), 294 (4400); IR ν_{max}^{KBr} cm^{-1} : 3200 br (OH), 1657 ($C=O$), 1625 ($CH_2=C-$), 1489, 1465, 1439, 1304, 1254 ($-O-$), 1177, 1065, 900, 783; NMR $\delta_{TMS}^{CDCl_3}$ ppm:

1.78 (3H, br s, $-CH_3$), 3.18 (2H, oct, $-CH_2-\overset{|}{CH}-$), 4.92 and 5.05 (1H each, br s, $CH_2=\overset{|}{C}-$), 5.32 (1H, t, $J=9Hz$, $-\overset{|}{CH}-CH_2-$), 6.44 (1H, d, $J=9Hz$, arom.), 7.77 (1H, d, $J=9Hz$, arom.), 10.60 (1H, s, OH, disappeared with D_2O); elemental analysis: Found: C, 65.58; H, 5.59. Calcd. for $C_{12}H_{12}O_4$: C, 65.44, H, 5.49%.

The molecular formula of I was assigned as $C_{12}H_{12}O_4$. Positive reaction of 2,4-dinitrophenylhydrazine and ferric chloride color tests for I, showed that the compound has the carboxyl and hydroxyl groups. Furthermore, in the IR spectrum of I, the carboxyl band at 1657 cm^{-1} shifted to the lower wave-number than usual absorption by the formation of internal hydrogen bonding. The ultraviolet absorption at 264 and 294 nm, the infrared absorption at 1657 cm^{-1} and the doublet at δ 6.44 and 7.77 in the NMR spectrum suggested the presence of the disubstituted hydroxyl benzoic acid system.

In the spin decoupling experiments, irradiation to the broad singlet at δ 1.78 sharpened the shape of the terminal methylene signal at δ 4.92. Irradiation to the triplet at δ 5.32 resulted in a change to the AB quartet of the octet splitting pattern for methylene signal at δ 3.18. These results revealed the presence of the system, $CH_2=C-\overset{|}{CH}-CH_2-$.

The methylation of I with etherial diazomethane gave colorless needles (Ia); mp 49°C, MS m/e : 234 (M^+); IR ν_{max}^{KBr} cm^{-1} : 3200 br, 1672, 1631, 1605, 1494, 1265, 1190, 1160, 908; NMR $\delta_{TMS}^{CDCl_3}$ ppm: 1.78 (3H, br s, $-CH_3$), 3.18 (2H, oct, $-CH_2-\overset{|}{CH}-$), 3.92 (3H, s, CH_3-O-), 4.92 and 5.05 (1H each, br s, $CH_2=\overset{|}{C}-$), 5.32 (1H, t, $J=9Hz$, $-\overset{|}{CH}-CH_2-$), 6.37 (1H, d, $J=9Hz$, arom.), 7.69 (1H, d, $J=9Hz$, arom.), 11.05 (1H, s, OH). The NMR spectrum showed that Ia was a monomethylester of I, and the residual phenolic hydroxyl group reacted with ferric chloride.

These data showed that the compound I corresponded to tubaic acid, which was obtained by the alkaline hydrolysis of rotenone by Kariyone *et al.*¹⁾ The IR spectrum of the compound I was identical with that of the authentic sample. The melting point was not depressed on admixture with the specimen. The specific rotation of the compound was the same as that of tubaic acid. Therefore the compound has the (R) configuration at the 2-position.²⁾

The compound II, colorless needles from *n*-hexane: mp 159~160°C, was optically inactive and also positive to 2, 4-dinitrophenylhydrazine and ferric chloride tests. The spectral data of II were as follows: MS m/e : 220 (M^+); UV λ_{max}^{EtOH} nm (ϵ): 248 (33600), 288 (4420), 315 (3850); IR ν_{max}^{KBr} cm^{-1} : 3000 br, 1670 ($C=O$), 1648, 1620, 1495, 1470, 1434, 1267, 1115, 1072, 899,

765; NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 1.40 (6H, s, geminate CH_3), 5.54 (1H, d, $J=10\text{Hz}$, $-\text{CH}=\text{CH}-$), 6.35 (1H, d, $J=9\text{Hz}$, arom.), 6.69 (1H, d, $J=10\text{Hz}$, $-\text{CH}=\text{CH}-$), 7.70 (1H, d, $J=9\text{Hz}$, arom.), 10.76 (1H, s, OH); elemental analysis: Found: C, 65.19; H, 5.53. Calcd. for $\text{C}_{12}\text{H}_{12}\text{O}_4$: C, 65.44; H, 5.49%.

These data suggested that the compound II was a structural isomer of I. In the NMR spectrum of II, the singlet signal (6H) at δ 1.40 showed the presence of a *gem*-dimethyl group. In the spin decoupling experiments, irradiation to the doublets at δ 5.54 and 6.35 altered to the singlet in the splitting pattern of the signals at δ 6.69 and 7.70, respectively. These data showed the presence of a disubstituted double bond which was conjugated to the tetrasubstituted aromatic ring.

The methylation of II with ethereal diazomethane gave colorless needles (IIa); mp $136\sim 139^\circ\text{C}$, MS m/e : 234 (M^+); IR $\nu_{\text{max}}^{\text{CCl}_4}$ cm^{-1} : 3000 br, 1670 ($\text{C}=\text{O}$), 1622, 1444, 1340, 1270, 1199, 1115, 994; NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 1.40 (6H, s, geminate CH_3), 3.87 (3H, s, $\text{O}-\text{CH}_3$), 5.54 (1H, d, $J=10\text{Hz}$, $-\text{CH}=\text{CH}-$), 6.31 (1H, d, $J=9\text{Hz}$, arom.), 6.69 (1H, d, $J=10\text{Hz}$, $-\text{CH}=\text{CH}-$), 7.60 (1H, d, $J=9\text{Hz}$, arom.), 11.18 (1H, s, OH). These spectral data and the ferric chloride test revealed that the monomethylester derivative was a phenolic compound.

The above evidence indicated the compound II to be β -tubaic acid which was obtained by the alkaline hydrolysis of deguelin by Takei *et al.*³⁾ The mixed mp of the compound II and authentic β -tubaic acid showed no depression, and the IR spectrum of II was

in full agreement with that of the authentic sample.

The compounds I and II were not artifacts of rotenone and deguelin, respectively, since the each compound was also identified by gas chromatography on the extract of the fresh plant with acetone or benzene at room temperature. The compounds inhibited the growth of *B. subtilis*, *Staphylococcus aureus* and *Escherichia coli* at high concentrations. The details of these data will be reported elsewhere.

It is noteworthy from the standpoint of biogenesis that tubaic acid and β -tubaic acid were firstly isolated as the natural products from the roots of *Derris elliptica* Benth.

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