ANTI-HUMAN IMMUNODEFICIENCY VIRUS PHENOLICS FROM LICORICE

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Five phenolics isolated from licorice inhibited the cytopathic activity of a human immunodeficiency virus. One of these a coumarin derivative named licopyranocoumarin, isolated from Xi-bei licorice, had structure 5, based on the chemical and spectroscopic evidence.

KEYWORDS—flavonoid; coumarin; licopyranocoumarin; licorice; Glycyrrhiza; antiviral substance; human immunodeficiency virus; cytopathic activity; OKM-1

A cell line named OKM-1, sensitive to the cytopathic activity of human immunodeficiency virus (HIV), was recently established from the peripheral blood of a patient with adult T-cell leukemia. Giant cells due to the cytopathic activity, were formed within a day on co-culture with HIV-infected Molt-4 cells (OKM-1 : Molt-4 = 3:1). As glycyrrhizin, the main component of licorice, reportedly inhibits the HIV-induced giant cell formation for Molt-4 cells, without reference to the effects of the other components, we have investigated the effects of these components of licorice on the cytopathic activity of HIV using OKM-1 cells.

In this experiment, we found that licochalcone A (1), isolicoflavonol (2), glycycomarin (3), glycyrrhisoflavone (4), and an additional component (5) of Xi-bei (Si-pei) licorice named licopyranocoumarin inhibited the giant cell formation at a concentration of 20 ug/ml without any observable cytotoxicity, while glycyrrhizin inhibited analogously at a concentration of 500 ug/ml. However, licochalcone B was cytotoxic even at this low concentration (20 ug/ml). Isoliquiritin and isoliquiritigenin had no great effect at this concentration.

The isolation of 5, which was not described in our recent publication, has been carried out as follows. The ethyl acetate extract of Xi-bei licorice was subjected to droplet countercurrent chromatography (DCCC) [chloroform-methanol-water (7:13:8, by volume), descending method], and then to column
chromatography over MCI-GEL CHP-20P. Further purification by preparative thin layer chromatography (silica gel) gave 5.

The compound 5, mp 137°C, [α]D +14° (c=1, acetone), forming yellow crystals, showed the ultraviolet (UV) spectrum [λ max MeOH: 211 (log ε 4.62), 262 (sh., 3.94) and 352 nm (4.21)] which is analogous to those of reported 3-arylcoumarins (e.g., glycyrrin (6)9 and glycyconarin (3)5,7). The high-resolution electron impact mass spectrum of 5 indicates the molecular formula C21H20O7 (found, 384.1175; calcld., 384.1209) for 5. The 1H nuclear magnetic resonance (NMR) spectrum (500 MHz, in acetone-d6) of 5 shows the signals due to a CH2-CH2 system [δ 2.89 (dt, J=17, 6 Hz, Hα-6), 2.82 (ddd, J=6, 9, 17 Hz, Hβ-6), 2.0 (in part overlaps with the solvent signals, Hα-7) and 1.82 (dt, J=14, 6 Hz, Hβ-7)], two 3H singlets [δ 3.91 (OCH3) and 1.31 (–C-CH3)], and the signals of a hydroxymethyl group [δ 3.63 (d, J=11 Hz) and 3.56 (d, J=11 Hz)], along with the signals assignable to the protons of the 3-arylcoumarin skeleton: Two 1H singlets [δ 7.97 (H-4) and 6.50 (H-10)] and the signals forming an ABX system [δ 6.42 (dd, J=2, 8 Hz, H-5'), 6.47 (d, J=2 Hz, H-3') and 7.21 (d, J=8 Hz, H-6')]. A nuclear Overhauser effect (11%) was observed for the H-4 signal at δ 7.97, when the methoxyl signal at δ3.91 was irradiated. This indicates that the methoxyl group should be at C-5 on the 3-arylcoumarin skeleton. Treatment of 5 with diazomethane afforded a methyleate (7), C23H24O7, mp 73°C, 1H-NMR (in CDCl3) δ3.84, 3.83 and 3.79 (3H each, s, 3 x OCH3). These signals indicate that the methyleate (7) has two additional methoxyl groups. Acetylation of 5 in the usual way afforded a triacetate (8), C27H26O10, mp 87°C, 1H-NMR (in CDCl3)
§2.29, 2.16 and 2.10 (3H each, s, 3 x OCOCH₃). Therefore, the 3-aryl-5-methoxycoumarin structure having two phenolic hydroxyl groups and an alcoholic hydroxyl group was assigned to 5.

Cyclization between the γ,γ-dimethylallyl group and the hydroxyl group at C-7 in glycyccoumarin (3) afforded a pyranocoumarin (9), C₂₁H₂₀O₆, mp 235°C (decomp.), whose ¹H-NMR spectrum closely resembles that of 5, except for the presence of the 6H singlet at δ 1.36 (the signal of the gem-dimethyl group) in the spectrum of 9, in place of the 3H singlet at δ 1.31 and the methylene protons of the hydroxymethyl group [at δ 3.56 and 3.63] in the spectrum of 5. Thus, licopyranocoumarin should be formulated as 7,8-dihydro-3-(2,4-dihydroxyphenyl)-8-hydroxymethyl-5-methoxy-8-methyl-2H,6H-benzo[1,2-b:5,4-b']dipyrene-2-one (5).¹⁰ The ¹H-NMR spectra of the esters of 7 with (R)- and (S)-forms of Mosher's acid¹¹ shows that 5 could be in part racemized (ca. 5%), if it was not racemized during the reactions (methylolation and esterification).

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REFERENCES AND NOTES

1) A part of this work was presented at the 108th Annual Meeting of the Pharmaceutical Society of Japan, Hiroshima, April 1988.
2) Identical with the cell line temporarily named ATL-1K.³
8) Licorice from the north-western region of China. Although the source plant is unidentified, Glycyrrhiza glabra L. var. grandiflora Reg. et Herd. is one of the generally accepted sources for the commercial material.⁷
10) According to a personal communication from Dr. M. Chen of Tsumura Laboratories, the same compound was isolated by him and his co-workers.

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