Communications to the Editor

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ANTI-HUMAN IMMUNODEFICIENCY VIRUS PHENOLICS FROM LICORICE[1)]

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Five phenolics isolated from licorice inhibited the cytopathic activity of a human immmunodeficiency 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

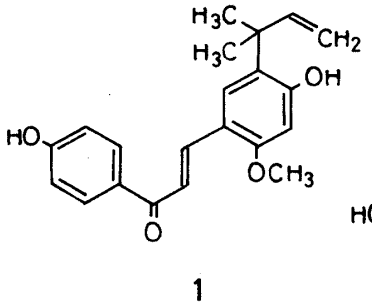
A cell line named OKM-1,[2,3)] sensitive to the cytopathic activity of human immunodeficiency virus (HIV), was recently established from the peripheral blood of a patient with adult T-cell leukemia.[3)] Giant cells due to the cytopathic activity, were formed within a day on co-culture with HIV-infected Molt-4 cells (OKM-1 : Molt-4 = 3:1).[3)] As glycyrrhizin, the main component of licorice, reportedly inhibits the HIV-induced giant cell formation for Molt-4 cells,[4)] 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**),[5)] isolicoflavonol (**2**),[6)] glycycoumarin (**3**),[6)] glycyrrhisoflavone (**4**),[7)] and an additional component (**5**) of Xi-bei (Si-pei) licorice[8)] named licopyranocoumarin inhibited the giant cell formation at a concentration of 20 μg/ml without any observable cytotoxicity, while glycyrrhizin inhibited analogously at a concentration of 500 μg/ml. However, licochalcone B[5)] was cytotoxic even at this low concentration (20 μg/ml). Isoliquiritin and isoliquiritigenin had no great effect at this concentration.

The isolation of **5**, which was not described in our recent publication,[7)] 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, $[\alpha]_D$ +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., glycyrin (**6**)[9] and glycycoumarin (**3**)[5,7]]. The high-resolution electron impact mass spectrum of **5** indicates the molecular formula $C_{21}H_{20}O_7$ (found, 384.1175; calcd., 384.1209) for **5**. The ^1H nuclear magnetic resonance (NMR) spectrum (500 MHz, in acetone-d_6) of **5** shows the signals due to a CH_2-CH_2 system [δ 2.89 (dt, J=17, 6 Hz, H_a-6), 2.82 (ddd, J=6, 9, 17 Hz, H_b-6), 2.0 (in part overlaps with the solvent signals, H_a-7) and 1.82 (dt, J=14, 6 Hz, H_b-7)], two 3H singlets [δ 3.91 (OCH_3) and 1.31 (-C-CH_3)], 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 δ 3.91 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 methylate (**7**), $C_{23}H_{24}O_7$, mp 73 °C, ^1H-NMR (in $CDCl_3$) δ 3.84, 3.83 and 3.79 (3H each, s, 3 x OCH_3). These signals indicate that the methylate (**7**) has two additional methoxyl groups. Acetylation of **5** in the usual way afforded a triacetate (**8**), $C_{27}H_{26}O_{10}$, mp 87 °C, ^1H-NMR (in $CDCl_3$)



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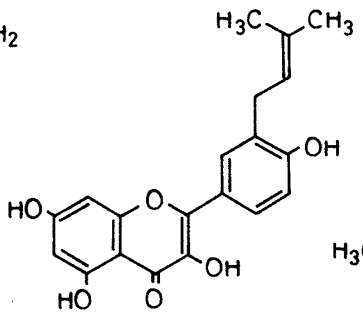

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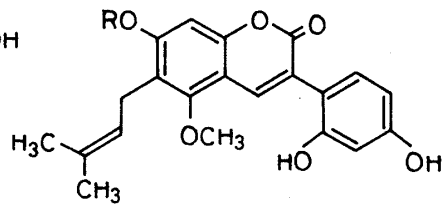
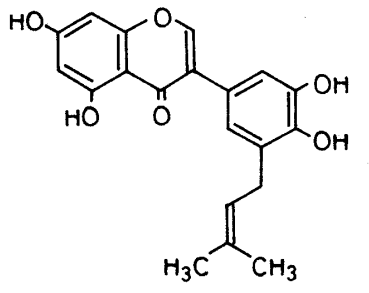


3: R=H
6: R=CH_3



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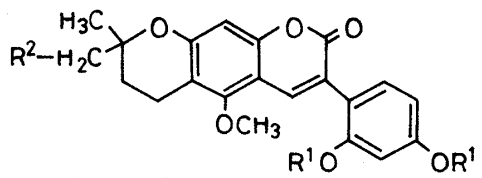

5: R^1=H, R^2=OH
7: R^1=CH_3, R^2=OH
8: R^1=$COCH_3$, R^2=$OCOCH_3$
9: R^1=R^2=H

δ2.29, 2.16 and 2.10 (3H each, s, 3 x $OCOCH_3$). 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 glycycoumarin (**3**) afforded a pyranocoumarin (**9**), $C_{21}H_{20}O_6$, mp 235 °C (decomp.), whose ^{1}H-NMR spectrum closely resembles that of **5**, except for the presence of the 6H singlet at δ1.36 (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']dipyrane-2-one (**5**).[10] The ^{1}H-NMR spectra of the esters of **7** with (R)- and (S)-forms of Mosher's acid[11] shows that **5** could be in part racemized (ca. 5 %), if it was not racemized during the reactions (methylation and esterification).

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REFERENCES AND NOTES

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