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ANTINEOPLASTIC AGENTS, 107. ISOLATION OF ACTEOSIDE AND ISOACTEOSIDE FROM *CASTILLEJA LINARIAEFOLIA*[1]

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ABSTRACT.—The southwestern Indian paintbrush, *Castilleja linariaefolia*, yielded extracts that displayed in vivo activity against murine P-388 (PS) lymphocytic leukemia. Separation guided by PS cell line inhibition led to isolation of cytotoxic compounds that were identified as the known glycosides acteoside [**1**] (ED_{50} 2.6 μg/ml) and isoacteoside [**2**] (ED_{50} 10 μg/ml). The identifications were established by spectral measurements and degradation studies. Mannitol was also found in this plant.

In mountainous areas of northern Arizona (2) and southern Utah, *Castilleja linariaefolia* Benth. is known as Indian paintbrush. While some 50 species of the large (3000 species and 220 genera) Scrophulariaceae family have been used in primitive cancer treatment, only one has been represented by the *Castilleja* genus (3), namely, the Mexican (Yucatan) *Castilleja communis* Benth. (4).

Extracts from *C. linariaefolia* gave confirmed activity against the Walker carcinosarcoma 256 (intramuscular WM system) in the rat. Each of the major plant parts appeared to contain the anticancer constituent(s), with the flowers showing the highest activity (90% inhibition of tumor growth at 266 mg/kg). When the murine P-388 lymphocytic leukemia (PS system) became available and a key fraction showed T/C 125–165% (10–40 mg/kg), we discontinued use of the WM system. [The PS in vitro studies were conducted in our laboratory according to procedures developed by the National Cancer Institute, and PS in vivo bioassays were performed under the auspices of the NCI (5).] Inconsistent results were obtained with both in vivo systems making fractionation difficult. Monitoring fractionation of *C. linariaefolia* with in vitro PS eventually yielded two of the PS cytostatic constituents. These were found to be the known glycosides acteoside (6–9) and isoacteoside (8). These caffeoyl glycosides have been isolated from a Labiatae species (8), and acteoside also occurs in a Gesneriaceae (7) and two Oleaceae (6,9) species; neither glycoside had hitherto been found in a plant of the Scrophulariaceae. The research was completed using a series of recently developed (10) experimental procedures augmented by dccc. The dccc technique has previously been used in the separation of iridoid glycosides from *Castilleja miniata* (11). Interestingly, dccc was the only effective method found for separation of myricoside, a bioactive substance closely resembling acteoside in structure (12). As part of the current study, D-mannitol also was isolated.

Acteoside [**1**] was identified on the basis of detailed spectral analyses (uv, ir, etc.) and by identification of alkaline and acid hydrolysis products and confirmed by comparison with an authentic sample provided by Professor I. Nishioka, Faculty of Pharmaceutical Sciences, Kyushu University. The general spectral features of isoacteoside [**2**] closely resembled those of acteoside, except that the L-rhamnose unit methyl group signal in the ^{1}H-nmr spectrum was shifted from δ 1.12 to 1.27 ppm. Also, the C-6 and C-3 D-glucose unit carbon signals in the ^{13}C-nmr spectrum were shifted from δ 62.43 to 64.70 and from 81.64 to 84.15

[1]For Part 106, see Nassimbeni *et al.* (1).

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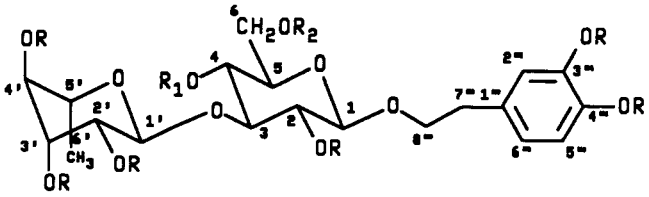
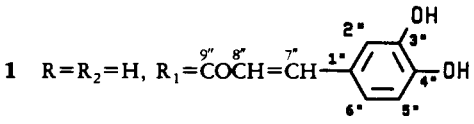
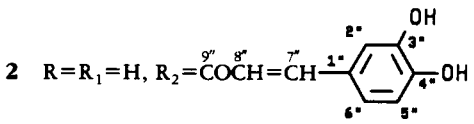




1 R=R_2=H, R_1=$^{9''}$COCH=$^{8''}$CH-$^{7''}$CH



2 R=R_1=H, R_2=$^{9''}$COCH=$^{8''}$CH-$^{7''}$CH

ppm, respectively. These data suggested an isomeric relationship, which was confirmed by a series of methylation, acetylation, and hydrolysis experiments (11,13,14).

Acteoside and isoacteoside exhibited moderate to weak cytotoxic activity in the PS in vitro system (ED_{50} 2.6 and 10 μg/ml, respectively). Because of the antibacterial (12) and cAMP phosphodiesterase inhibitory (15) activity shown by several closely related natural products, these glycosides are worthy of further biological study.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Paper partition chromatography (ppc) was performed by the ascending method using Toyo Roshi No. 50 paper and *n*-BuOH–HOAc–H_2O (4:1:2). Mp's were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Ir were recorded with an Hitachi EPI-G2 spectrometer. The ^{1}H-nmr spectra were measured with an Hitachi R 40 spectrometer at 90 MHz and the ^{13}C-nmr spectra with a JEOL JNM FX-200 spectrometer at 50.3 MHz. Chemical shifts are given in ppm (δ) downfield from TMS as an internal standard. Solution phase sims (16) mass spectra were obtained using a Varian MAT 312 spectrometer equipped with a modified capillaritron source and 0.14 M NaI in sulfolane as liquid phase. The eims were recorded using an Hitachi M-70 spectrometer.

PLANT MATERIAL.—The recollection (original in August 1966, Kaibab, N.F., Arizona) of *C. linariaefolia* (ca. 500 kg dry wt), used here was made in August 1978, in the Dixie National Forest, Garfield Co., Utah, at an elevation of 7000–8000 ft. Taxonomic identification was made by one of us (CPP) in the USDA Laboratory and by E. Lehto in the Department of Botany at Arizona State University. Herbarium specimens are maintained in the Department of Botany and in the Cancer Research Institute at Arizona State University.

PLANT EXTRACTION.—Dried plant material (leaves, stems, and roots, 36 kg) from the 1978 collection was extracted with a mixture (160 liters) of CH_2Cl_2 and MeOH (1:1) (10) at ambient temperature for 3 weeks. The extract was separated into CH_2Cl_2 and H_2O phases on addition of 25% by volume of H_2O. The aqueous phase was adjusted by the addition of MeOH and CH_2Cl_2 to achieve a 4:1:2 ratio for H_2O-MeOH-CH_2Cl_2; the plant was extracted with this mixture for 7 weeks. Addition of 15% by volume of H_2O resulted in separation of the CH_2Cl_2 phase which was combined with that obtained from the first partition. Concentration gave a CH_2Cl_2 extract (712 g; PS in vitro ED_{50} 19 μg/ml; PS in vivo inactive at 12.5–100 mg/kg). The H_2O phase was concentrated to give an extract (3.5 kg) that was marginally active (in vivo T/C 120% at 25 mg/kg; PS in vitro ED_{50}>100 μg/ml).

SOLVENT PARTITION SEQUENCE.—A portion of the H_2O extract (180 g) was successively partitioned between MeOH-H_2O (9:1) (1800 ml)→(4:1)→(1:1) with hexane (3 × 1800 ml), CCl_4 (3 × 1800 ml), and CH_2Cl_2 (3 × 1800 ml), respectively. Concentration of the partitioned fractions gave hexane (1.5 g; PS in vitro ED_{50} 6 μg/ml), CCl_4 (1.2 g; PS in vitro ED_{50} 24 μg/ml), CH_2Cl_2 (4.6 g; PS in vitro ED_{50} 51 μg/ml), and H_2O (142.8 g; PS in vitro ED_{50} 36 μg/ml) fractions. None of these fractions exhibited activity in the PS in vivo system when tested at dose levels of 3.12–25 mg/kg. However, in a number of earlier experiments, fractions had been obtained at this stage showing T/C 165 at 40 mg/kg.

ISOLATION OF ACTEOSIDE [1] AND ISOACTEOSIDE [2].—An aliquot (10.22 g) of the H_2O fraction was chromatographed on Sephadex LH-20 (805 g; 77 × 8 cm) using MeOH-H_2O (4:1) as eluent. Fractions were monitored by ppc. After elution of 5.1 g of material, a fraction (2 g) was obtained from which D-mannitol (0.252 g) was isolated as colorless needles, mp 171–172°, and found to be identical (ir, tlc) with an authentic sample. Further elution gave 1.8 g of material (inactive against PS in vitro), followed by a fraction (1.3 g) that was active in vitro (ED_{50} 4.1 μg/ml, PS in vivo inactive at 3.12–25 mg/kg). A 1.94-g portion of the in-vitro-active fraction (obtained after repeating the Sephadex LH-20 chromatographic step) was treated with MeOH. The soluble fraction (1.38 g) was dissolved in a minimum volume of the upper layer prepared from $CHCl_3$-MeOH-H_2O (5:5.7:3) and placed in the transfer tube of a dccc apparatus filled with the upper layer of the same solvent system as stationary phase. The flow rate of the moving lower phase was 0.66 ml/min. Three fractions were collected on the basis of monitoring by ppc.

Acteoside [1] (0.388 g) was isolated as a pale yellow amorphous powder from the third fraction. The first fraction (0.174 g) was again subjected to dccc to give isoacteoside [2] (0.107 g) as a light brown amorphous powder. Acteoside [1] exhibited the following physical properties: mp 145–149° [lit. (6) mp 147–150°]; solution phase sims *m/z* $[M+H]^+$ 625 (21%), 480 (10%), 472 (24%), $[M+H-$caffeoyl-3,4-dihydroxyphenethyl$]^+$ 325 (100%). The identity was confirmed by direct comparison of the ^1H- and ^{13}C-nmr spectra with those of an authentic sample. Treatment of acteoside with Ac_2O/pyridine followed by Si gel cc gave the peracetate as a colorless amorphous powder: uv (MeOH) λ max 283 (log ε 4.19) nm; the cd spectrum was identical with that already published (12). Isoacteoside [2] exhibited mp 136–139° and solution phase sims *m/z* 625 $[M+H]^+$ (22%), 480 (29%), 472 (30%), 325 $[M+H-$caffeoyl-3,4-dihydroxyphenethyl$]^+$ (100%). Acetylation and chromatography of the product gave the peracetate as a colorless amorphous powder: uv (MeOH) λ max 280 (log ε 4.53) nm. Acteoside [1] and isoacteoside [2] both showed activity against the PS cell line (ED_{50} 2.6 and 10 μg/ml, respectively).

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