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ISOLATION AND CHARACTERIZATION OF A NEW TRITERPENE FROM *IRIS MISSOURIENSIS*[1]

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Iris missouriensis Nutt. (Iridaceae) is indigenous to North America and widely distributed along the west coast. The whole plant has been extensively used by American Indians for medicinal purposes (1). Prompted by the discovery of potential antitumor compounds from other *Iris* species (e.g., irisquinone) (2), we have similarly investigated *I. missouriensis*. In earlier studies, using the roots of *I. missouriensis*, two novel quinones (irisoquin and deoxyirisoquin) (3), a novel triterpene (missourin) (4), and two cytotoxic triterpenes (isoiridogermanal and zeorin) (4) were isolated and characterized.

In continuing our search for potential anticancer drugs from this plant, mangiferin, 7β-hydroxystigmasterol, 7β-hydroxysitosterol, 7-oxostigmasterol, 7-oxositosterol, and betulinic acid were isolated. The structures of these compounds were determined by spectral analyses and confirmed by comparison with authentic samples or published data.

In addition, a novel triterpene was isolated and given the trivial name missouriensin (**1**). The mass spectrum showed **1** to have a molecular ion (*m/z* 484) corresponding to $C_{32}H_{52}O_3$. Furthermore, a close structural relationship between **1** and zeorin acetate (**3**) was suggested by comparison of ^{13}C-nmr spectra, and the ^{1}H-nmr angular methyl signals of **1-4**. As indicated by the ^{1}H-nmr and ^{13}C-nmr spectra, only seven angular methyls were present in **1** as opposed to the eight methyl signals demonstrated by compounds **2** (zeorin) and **3**. The two singlets at δ 1.16 and 1.12, corresponding to the C_{29} and C_{30} methyls of **3**, were absent in the ^{1}H-nmr spectrum of **1**. However, the ^{1}H-nmr spectrum of **1** revealed two singlets at δ 4.88 and 5.03 (each intergrating for one proton) and a broad singlet at δ 1.85 (3H), suggesting the presence of a terminal methylene moiety and one olefinic methyl group. The presence of this vinylidene group was further supported by ir absorption bands at 3100, 1650, and 880 cm^{-1}. These spectral data suggested a terminal methylene was situated at the C_{22}-C_{29} position.

The ir spectrum of **1** also showed strong absorption bands at 3485 and 1735 cm^{-1} that are associated with hydroxy and ester groups, respectively. Also, the presence of an acetate group was indicated by the broad singlet at δ 2.02 (3H). The location of the acetate group at the 6 position of compound **1** was confirmed by the doublet of a triplet signal (1H) of the methine proton with coupling constants of 11 and 7.2 Hz at δ 5.21 (4).

The 21 β-H signal of zeorin acetate (**3**) and its dehydration product (**4**) appeared as quartets at δ 2.22 and 2.86, respectively. Thus, the absence of this signal in the ^{1}H-nmr of compound **1** suggested that the OH group was located at the 21-β position. In order to confirm the structure of **1**, chemical conversion of zeorin was performed as shown in Scheme 1. Dehydration of zeorin acetate (**3**) with $POCl_3$ afforded a mixture of two compounds (**4** and **5**) (5), that were separated by chromatography over $AgNO_3$ impregnated silica gel utilizing

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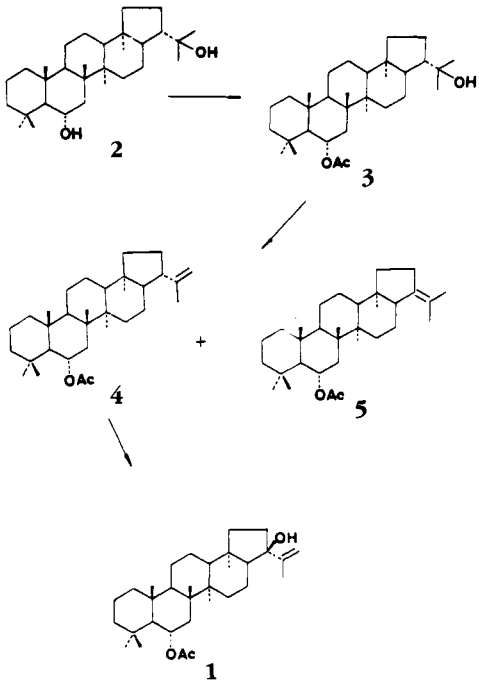

SCHEME 1

n-hexane-C_6H_6 (5:1) as the eluent. Oxidation of compound **4** over SeO_2 gave one major product, and this was in every aspect identical to compound **1**. Hence, the structure of missouriensin (**1**) was established as 6α-acetoxyl-21 β-hydroxylhop-22(29)-ene.

Evaluation of the cytotoxic potential of these isolates with cultured P-388 cells showed that only 7β-hydroxystigmasterol and 7β-hydroxysitosterol were active, having ED_{50} values of 0.1 and 0.2 μg/ml, respectively.

EXPERIMENTAL

GENERAL.—Melting points were determined by means of a Kofler hot-plate apparatus and are uncorrected. Specific rotations were measured on a Perkin-Elmer 241 polarimeter. The uv spectra were obtained with a Cary Model 118 spectrophotometer, and ir spectra with a Nicolet MX-1 ft-ir spectrophotometer. ^{1}H-nmr and ^{13}C-nmr spectra were recorded with a Nicolet NT-1280 spectrometer operating at 360 or 200 MHz, and 90 MHz, respectively. TMS was used as an internal standard, and chemical shifts are reported on the ppm scale. Hplc was performed with a system comprised of Beckman 110A and 100A pumps, a Perkin-Elmer LC-85 ultraviolet detector, a Beckman 421 system controller, and an Altex C-R1A printer-plotter. Low-resolution mass spectra were obtained with a Varian MAT-112 double focussing mass spectrometer.

PLANT MATERIAL.—The roots of *I. missouriensis* were collected in Nevada during July 1981 and identified by the staff of the Economic Botany Laboratory, BARC-East, USDA, Science and Education Administration, Beltsville, MD. A voucher specimen representing this collection has been deposited in the Herbarium of the National Arboretum, Washington, DC.

EVALUATION OF CYTOTOXIC ACTIVITY.—Cytotoxic activity was assessed utilizing cultured P-388 cells, according to protocols of the National Cancer Institute (6), as described previously (7).

EXTRACTION AND ISOLATION.—Extraction of the root of *I. missouriensis* has been described previously (3,4).

ISOLATION OF MANGIFERIN.—The MeOH extract deposited a yellowish-orange precipitate which, following repeated crystallization from MeOH and 50% aqueous EtOH, gave mangiferin (123 mg, 0.0135%); mp 269-271°; $[\alpha]^{25}D$ +38 (c 0.21, pyridine) (8). The compound was confirmed to be mangiferin by comparison with an authentic sample.

ISOLATION OF PHYTOSTEROLS.—Chromatography of the $CHCl_3$ extract (29 g; ED_{50}, 0.26 μg/ml) on silica gel (660 g) eluting successively with $CHCl_3$ and $CHCl_3$/MeOH mixtures afforded 20 fractions (2 liters each). Fractions 6 to

14 (total weight of 14.2 g; ED_{50} values ranging from 0.23 to 2.7 μg/ml), eluted with 1-10% MeOH in $CHCl_3$, were separately subjected to repeated chromatography.

Rechromatography of fractions 6 to 10 over silica gel [eluting with n-hexane-Me_2CO (9:2) and n-hexane-Me_2CO (9:1)] gave a mixture of 7β-hydroxystigmasterol and 7β-hydroxysitosterol, and a mixture of 7-oxostigmasterol and 7-oxositosterol, respectively. These mixtures were separated by hplc employing a μ-Bondapak C_{18} column (8mm ID×30 cm, Waters). Monitoring absorbance at 214 nm, with H_2O-MeCN (5:95) as the eluent (flow rate, 2 ml/min), 7β-hydroxystigmasterol (retention time, 34.95 min), 7β-hydroxysitosterol (retention time, 39.62 min), 7-oxostigmasterol (retention time, 27.03 min), and 7-oxositosterol (retention time, 30.17 min), were obtained.

7β-HYDROXYSTIGMASTEROL.—Crystallized from a mixture of $CHCl_3$/MeOH as colorless needles (13 mg, 0.0013%); mp 154-157°; $[\alpha]^{25}D$ −28° (c 0.036, $CHCl_3$). The compound was identified as 7β-hydroxystigmasterol by comparison with published data (9-11).

7β-HYDROXYSITOSTEROL (EPI-IKSHUSTEROL).—Crystallized from a mixture of $CHCl_3$-MeOH as colorless needles (12 mg, 0.0012%); mp 209-212°; $[\alpha]^{25}D$ −25.9° (c 0.02, $CHCl_3$). The compound was identified as epi-ikshusterol by comparison with published data (12,13).

7-OXYSTIGMASTEROL.—Crystallized from a mixture of $CHCl_3$/MeOH as colorless needles (8 mg, 0.008%); mp 158-161°; $[\alpha]^{25}D$ −76° (c 0.03, $CHCl_3$). The compound was identified as 7-oxostigmasterol by comparison with published data (14).

7-OXOSITOSTEROL.—Crystallized from a mixture of $CHCl_3$-MeOH as colorless needles (6.5 mg, 0.00065%); mp 152-154°; $[\alpha]^{25}D$ −83° (c 0.025, $CHCl_3$). The compound was identified as 7-oxositosterol by comparison with published data (14).

ISOLATION OF BETULINIC ACID.—Rechromatography of fractions 11 to 14 over silica gel eluting with n-hexane-EtOAc-HCOOH (10:1:0.01) gave a glassy-like solid which, on crystallization from MeOH, gave colorless needles (30 mg, 0.003%); mp 296-298° (15); $[\alpha]^{25}D$ +7.2 (c 0.06, pyridine). The compound was identified as betulinic acid by comparison with an authentic sample (15).

ISOLATION OF MISSOURIENSIN (**1**).—Rechromatography of fractions 6 to 10 over silica gel with 1.0% MeOH in $CHCl_3$ gave impure **1** (35 mg). This was purified by repeated chromatography over silica gel (15 g) eluting with hexane-Me_2CO (97:3) and then hexane-EtOAc (99:1) (3.5 mg; 0.00035%); mp 213-216°; ir (KBr) ν max 3485, 3100, 1735, 1650, 1249, 889 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) 5.21 (dt, J=11, 7.2 Hz, 1H), 5.03 (br s, 1H), 4.88 (br s, 1H), 2.02 (s, 3H), 1.85 (s, 3H), 1.09 (s, 3H), 1.01 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.84 (s, 3H), 0.68 (s, 3H); ^{13}C nmr (90 MHz, $CDCl_3$) 39.5 (t, C-1), 18.2 (t, C-2), 40.7 (t, C-3), 33.0 (t, C-4), 58.2 (d, C-5), 71.9 (d, C-6), 40.0 (t, C-7), 42.5 (s, C-8), 49.1 (d, C-9), 39.3 (s, C-10), 20.7 (t, C-11), 23.2 (t, C-12), 49.6 (s, C-13), 41.9 (s, C-14), 33.3 (t, C-15), 20.2 (t, C-16), 64.1 (d, C-17), 44.4 (s, C-18), 43.3 (t, C-19), 37.0 (t, C-20), 83.5 (s, C-21), 149.1 (s, C-22), 36.1 (q, C-23), 22.0 (q, C-24), 16.9 (q, C-25), 17.7 (q, C-26), 16.4 (q, C-27), 15.2 (q, C-28), 112.0 (t, C-29), 20.9 (q, C-30), 170.1 (s, acetyl carbonyl carbon), 22.0 (q, acetyl methyl carbon) ppm; ms (70 eV) *m/z* 484 (M^+), 466, 424, 297, 271, 257, 203, 191.

CHEMICAL CONVERSION OF ZEORIN (**2**) TO COMPOUND **1**.—The synthesis of missouriensin (**1**) from zeorin (**2**) is summarized in Scheme 1.

PREPARATION OF 6α-ACETOXY-ZEORIN (**3**).—Zeorin (80 mg) was dissolved in a solution containing 5 ml pyridine and 4 ml Ac_2O, and the mixture was left overnight at room temperature. Usual work up of the reaction mixture gave 82.1 mg of 6-acetoxyl-zeorin; mp 223-225°; ir (KBr) ν max 3600, 2995, 1713, 1249 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) 5.22 (dt, J=11.5, 7.2 Hz, 1H), 2.04 (s, 3H), 1.21 (s, 3H), 1.16 (s, 3H), 1.09 (s, 3H), 1.03 (s, 3H), 0.97 (s, 3H), 0.85 (s, 3H), 0.76 (s, 3H); ms (70 eV) *m/z* 486 (M^+).

DEHYDRATION OF ZEORIN MONOACETATE (**3**).—Compound **3** (80 mg) was dissolved in 25 ml of dry pyridine. About 1 ml $POCl_3$ was added dropwise, with stirring, over a period of 15 min. After stirring at room temperature for 3 h, the reaction was stopped by pouring the mixture into ice cold H_2O. A crystalline precipitate was obtained (58 mg) and chromatographed over $AgNO_3$ impregnated silica gel (20 g) developing with hexane-C_6H_6 (1:5). This afforded compound **5** (25 mg); mp 121-123°; ir (KBr) ν max 1738, 1250 cm^{-1}; ^1H nmr (200 MHz, $CDCl_3$) 5.17 (td, J=11, 7 Hz, 1H), 2.02 (s, 3H), 0.86 (s, 3H), 0.58 (s, 3H); ms (70 eV) *m/z* 468 (M^+), 426 (M^+-CH_2CO). Following compound **5** in the elution profile, compound **4** was obtained (18 mg); mp 198-201°; ir (KBr) ν max 3080, 1740, 1643, 1253, 890 cm^{-1}, ^1H nmr (360 MHz, $CDCl_3$) 5.23 (dt, J=7.2, 11 Hz, 1H), 4.78 (br s, 2H), 2.68 (q, J=7.2 Hz, 1H), 2.03 (s, 3H), 1.75 (s, 3H), 1.10 (s, 3H), 1.03 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H), 0.85 (s, 3H), 0.72 (s, 3H) δ; ms (70 eV) *m/z* 468 (M^+), 426 (M^+-CH_2CO).

OXIDATION OF 6α-ACETOXY-21 βH-HOP-22(29)-ENE (**4**).—A mixture of compound **4** (15

mg), freshly sublimed Se_2O (4 mg), and dry pyridine (0.15 ml) in 95% EtOH (5 ml) was heated under reflux for 3 h. The solvent was removed in vacuo, and the residue was chromatographed over silica gel eluting with $CHCl_3$ to afford 6α-acetoxyl-21β-hydroxy-hop-22(29)-ene (9.5 mg). Comparison of mp (213-215°), ^1H nmr, ^{13}C nmr, ms and ir, indicated that this compound was identical to compound **1**.

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