Journal of Natural Products
Vol. 58, No. 1, pp. 68-73, January 1995

OXIDATION AND GLUCOSE CONJUGATION OF SYNTHETIC ABIETANE DITERPENES BY *CUNNINGHAMELLA* SP. II.[1] NOVEL ROUTES TO THE FAMILY OF DITERPENES FROM *TRIPTERYGIUM WILFORDII*

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ABSTRACT.—Abietane diterpenes from the perennial herb, *Tripterygium wilfordii*, have been shown to possess antiinflammatory activity. To obtain novel analogues of these diterpenes, two synthetic diterpenes, isotriptophenolide [**1**] (12,19-dihydroxy-18(4→3)*abeo*-abieta-3,8,11,13-tetraen-18-oic acid lactone) and triptophenolide [**3**] (14,19-dihydroxy-18(4→3)*abeo*-abieta-3,8,11,13-tetraen-18-oic acid lactone) were incubated with the filamentous fungi, *Cunninghamella echinulata* and *C. elegans*. The structures of the metabolites were then determined by spectroscopic methods. Both species of *Cunninghamella* glucosidated **1** at C-12 to yield **2**. When incubated with triptophenolide [**3**], *C. elegans* and *C. echinulata* produced hydroxylated [**6**] and glucosylated [**7**] metabolites. In addition to B-ring hydroxylation, aromatic hydroxylation at ring C was also observed. Both species hydroxylated **3** to yield the dihydrodiol **4** which autooxidized to the quinone **5**.

Extracts of the perennial herb *Tripterygium wilfordii* Hook f. (Celastraceae) have been shown to possess antitumor and anti-inflammatory activity in a variety of in vivo and in vitro models (1–4). Attempts to identify the pharmacologically active compounds from crude extracts resulted in the isolation of the abietane diterpene epoxides, triptolide and tripdiolide by Kupchan in 1972 (4). Several other novel diterpenes and triterpenes from *T. wildfordii* have subsequently been identified (5–9). Recently, seven novel quinoid diterpenes were isolated from dried stalks of this plant (10,11). These triptoquinones are of clinical interest as they may be responsible in part for the anti-inflammatory effects of the crude plant extracts; micromolar quantities have been shown to inhibit the release of the important inflammatory mediators, interleukins 1α and 1β from lipopolysaccharide-stimulated human peripheral monocytes (10–12).

Previous studies in our laboratory have focussed on the production of novel analogues of the *T. wilfordii* abietane diterpenes using a combination of chemical synthesis and microbial transformation. The filamentous fungi, *Syncephalastrum racemosum*, *Aspergillus fumigatus*, and *Cunninghamella* sp. were shown to hydroxylate the B ring and/or the isopropyl side-chain of a synthetic lactone diterpene precursor, 19-hydroxy-18(4→3)*abeo*-abieta-3,8,11,13-tetraen-18-oic acid lactone (13,14). In this study, the oxidation of isotriptophenolide [**1**] (12,19-dihydroxy-18(4→3)*abeo*-abieta-3,8,11,13-tetraen-18-oic acid lactone) and triptophenolide [**3**] (14,19-dihydroxy-18(4→3)*abeo*-abieta-3,8,11,13-tetraen-18-oic acid lactone) by *Cunninghamella echinulata* and *C. elegans* was investigated.

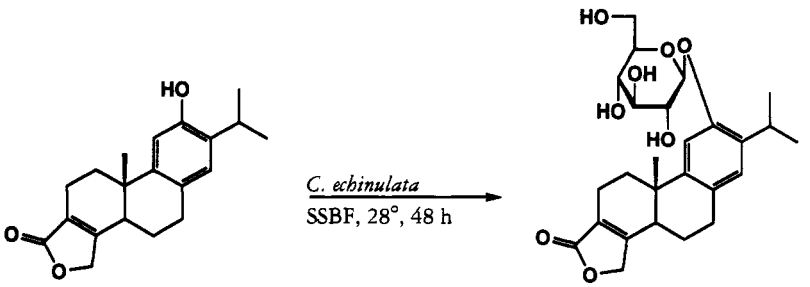
RESULTS AND DISCUSSION

In contrast to bacteria, fungi do not use diterpenes as a carbon source and therefore

[1]For part 1 in this series, see Milanova *et al.* (14).

fungal oxidation is not followed by ring cleavage and degradation of the compound of interest (15,16). Thus, the microbial transformation of isotriptophenolide [**1**] and triptophenolide [**3**] was studied in 15 strains of yeast and filamentous fungi. Only the following strains were found to metabolize compounds **1** and **3**: *Cunninghamella elegans* var. *chibaensis* Kuwabara et Hoshino and *C. echinulata* (Thaxter) Thaxter var. *echinulata*. None of the yeasts tested were capable of oxidizing compounds **1** and **3**.

Scheme 1 depicts the metabolism of **1** by *C. echinulata*. Isotriptophenolide [**1**] (0.1 mg/ml) was added to 48-h old cultures of *C. echinulata* (see Experimental) and incubation for a further 48 h resulted in the production of 12-glucosyl-isotriptophenolide [**2**]. *C. elegans* also metabolized **1** under the same growth conditions and produced one polar



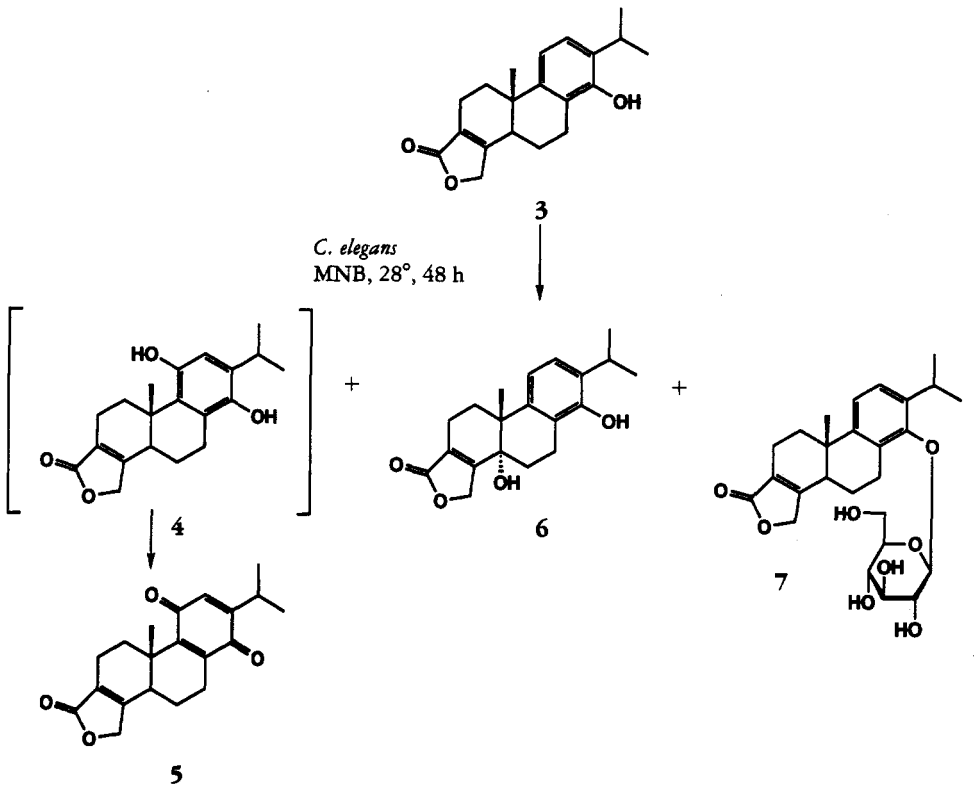
1 **2**

SCHEME 1. Biotransformation of the isotriptophenolide **1** by the fungus, *C. echinulata*. The starting material [**1**] (180 mg–0.1 mg/ml) was dissolved in EtOH and added to the 48-h culture and incubated for a further 48 h. The polar metabolite was identified as outlined in the Experimental.

metabolite which was identical to **2** when extracts were examined by tlc (data not shown).

Incubation of stationary phase cultures of *C. elegans* with triptophenolide [**3**] for 48 h yielded three products which were extracted from the cell suspension and separated by flash chromatography on Si gel. Scheme 2 presents the structures of the triptophenolide metabolites produced by *C. elegans*. Whereas the major route of metabolism of isotriptophenolide [**1**] was β-glucosyl conjugation, triptophenolide [**3**] oxidation was primarily an aromatic hydroxylation (35%) yielding compound **5**. Only 5% of the starting material [**3**] was glycosylated. The dihydrodiol **4** was not isolated from the extracts as it rapidly converted to the quinone **5**. The benzoquinone **5** is structurally related to the anti-inflammatory triptoquinones with the exception that it is a butenolide whereas the triptoquinones have a carboxylic acid group attached to ring A (10–12). The glucosyl conjugate **7** and the 5α,14-diol **6** are novel compounds. Pertinent ^{1}H-nmr and ms data for the metabolites [**5–7**] isolated from *C. elegans* are noted below. The metabolism of **3** by *C. elegans* was unaffected by changing the growth medium from MNB to SSBF (see Experimental). Moreover, incubation of *C. echinulata* with **3** produced the same pattern of polar metabolites when cell extracts were separated by tlc (data not shown) suggesting that both species were capable of producing compounds **5–7**.

Glucosides **2** and **7** showed the ($[M+NH_4]^+$) peak at *m/z* 492 in the ci mass spectrum which was 180 mass units higher than compounds **1** and **3**. The molecular formula of both compounds ($C_{26}H_{34}O_8$) was found by the hrcims peaks at *m/z* 475.2337 and 475.2339 ($[M+H]^+$). They showed uv maxima for **2** at 220 and 276 nm and for **7** at 217 and 270 nm, and ir bands for **2** at 3486 (OH), 2632 (aromatic CH), and 1763 (C=O) cm^{-1}, and for **7** at 3608 (OH), 3005 (aromatic CH), and 1713 (C=O) cm^{-1}.



SCHEME 2. The biotransformation of the triptophenolide **3** by *C. elegans*. The starting material [**3**] (200 mg, 0.1 mg/ml) was added to 48-h cultures of *C. elegans*. After 48 h, polar metabolites were extracted and identified as outlined in the Experimental.

The ^{1}H-nmr spectra of **2** and **7** resembled those of **1** and **3** except for the additional signals for the glucose unit, which are in the expected range of δ 3.17 to 4.60. The characteristic anomeric proton of the sugar was seen as a doublet (J=7 Hz) at δ 4.93 [**2**] and δ 4.71 [**7**]. The dihedral angle between the anomeric and the adjacent protons (the angle between H-1′ and H-2′ in the glucose unit) is 150°. It was determined by use of the Karplus curve and provides evidence in support of the β-anomeric configuration for both glucosides. The signals corresponding to the hydroxyl groups in the sugar moiety were exchangeable with D_2O.

Compound **5** showed a molecular ion peak at *m/z* 326 in the ms which was 14 mass units higher than **3**. The molecular formula $C_{20}H_{22}O_4$ was determined by hreims (*m/z* 326.1523, M^+). Compound **5** showed ir absorptions at 2929 (aromatic CH), 1756, and 1680 (C=O) cm^{-1} and a uv max at 261 nm due to the quinone chromatophore. The ^{1}H- and ^{13}C-nmr spectra of **5** showed signals for a quinone unit (δ_H 6.40, δ_C 131.6, 153.4, 187.1 and 187.4), an isopropyl group (δ_H 1.10 and 3.00; δ_C 21.2, 21.2, and 26.4), a tertiary methyl (δ_H 1.14, δ_C 18.3), a double bond (δ_C 125.4 and 161.3), five methylenes (δ_C 18.6, 24.7, 30.7, 30.8, and 70.1), and one quaternary carbon (δ_C 36.6). The structure of **5** was compared with an authentic sample obtained by synthesis. Quinone **5** was generated as a minor oxidation product in the synthesis of racemic triptolide and triptonide by Lai *et al.* (17).

Metabolite **6** showed a molecular ion peak at *m/z* 328, which was 16 mass units higher than the parent compound **3**. Its molecular formula, $C_{20}H_{24}O_4$, was obtained by hreims (*m/z* 328.1675, M^+). The ir spectrum of this compound showed absorption bands

for hydroxyl groups at 3607 and 3560 cm^{-1} while the ^{1}H-nmr spectrum was similar to that of **3**. However, the absence of the H-5 proton in the spectrum and the presence of a new hydroxy proton at δ 1.90, which was exchangeable with D_2O, suggested that **6** was a C-5 hydroxy analogue. The H-19 protons were also shifted downfield from δ 4.77 to 4.90 and revealed a wider splitting, thereby further confirming that the new hydroxyl group is at C-5. The C-20 methyl is located at δ 1.09 and the isopropyl methyls are found at δ 1.25 and 1.27. The signal of the C-20 angular methyl group was seen at higher field than the isopropyl methyl groups which suggested a trans junction of the A and B rings and therefore that the C-5 hydroxyl was α oriented. The COSY nmr spectrum showed that the H-19 protons were coupled with two H-2 protons at δ 2.39 (1H, m) and 2.55 (1H, br d, J=16 Hz) (homoallylic coupling). The H-7 protons were located between δ 2.80 and 3.02 (2H, m), and showed cross-peaks to one multiplet between δ 1.98 and 2.15 (2H, m), and one proton at δ 2.23 (1H, ddd, J=9.9 and 14 Hz). This indicates that one H-6 proton is situated in the multiplet and the other is the one at δ 2.23.

Irradiation of the C-20 methyl resulted in enhancement of the H-2 proton signal at δ 2.39, a signal at δ 2.31 (1H, dd, J=6 and 13 Hz) and the H-6 proton signal at δ 2.23, thereby suggesting that these protons are Hβ-2, Hβ-1, and Hβ-6, respectively. Irradiation of the Hβ-1 proton increased signal intensities of the H-11 signal at δ 6.91 (1H, d, J=8 Hz) as expected, and one proton in the multiplet between δ 1.98 and 2.15, which indicated that the remaining proton in that multiplet is Hα-1. The above results were in agreement with structure **6**.

Filamentous fungi have been shown to possess great versatility in the substrates they can oxidize. For example, *Cunninghamella* spp. alone have been shown to perform regio- and steroselective oxidations of such chemically diverse substrates as warfarin (18), biphenyl (19), plant steroids (20), furosemide (21), fluoranthene (22), and *N*-methylcarbazole (23). The data obtained in this study and the results of previous work with structurally related abietane diterpenes (14,15) indicate that both B- and C-ring oxidation of abietane diterpenes can be accomplished using *Cunninghamella* spp.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Radial chromatography was performed using a Chromatotron Model 8924 with 1-mm Si gel 60 plates (PF 254) containing gypsum. Mps were determined using a Kofler block melting-point apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 141 automatic polarimeter in $CHCl_3$ solution using a quartz cell of 10-cm path length with the concentration (in g/100 ml) given in parentheses. The ir were recorded on Perkin-Elmer 710, 710B, and 1710 spectrometers in $CHCl_3$ or Me_2CO solution (using NaCl cells of 0.1 mm path length) or as a thin film (using NaCl plates). The uv spectra were recorded on a Unicam SP800 spectrometer using quartz cells of 1-cm path length. The ^{1}H-nmr spectra were determined on Bruker WH-400, AE-200, or Varian XL-300 spectrometers and in the solvents given in parentheses. The chemical shifts are reported in ppm relative to TMS. Assignments, where given, are based on a combination of chemical shift, coupling constant, decoupling, and nOe difference data. The ^{13}C-nmr spectra were recorded on Bruker AE-20 or Varian XL-300 spectrometers and the chemical shifts are reported in ppm relative to TMS. The eims were recorded on AEI-MS-9 or Kratos-MS-50 (low- and high-resolution analyses) or Delsi Nermag R10-10C (cims) spectrometers.

FUNGAL MATERIAL.—The following strains were able to oxidize and glucosylate **1** and **3**: *Cunninghamella echinulata* (ATCC# 9244) and *Cunninghamella elegans* var. *chibaensis* Kuwabara et Hoshino (ATCC# 20230).

GROWTH CONDITIONS.—Fungi were stored on potato dextrose agar slants under sterile mineral oil. Erlenmeyer flasks were inoculated with 1 ml of spore suspension (2×10^8 spores/ml) prepared from 7 slants in distilled H_2O to obtain a final concentration of 2.5×10^6 spores/ml. The ratio of flask volume to medium volume was 5:1. *C. echinulata* spores were inoculated into 1.8 liters of SSBF medium (see below) containing 2% glucose and the culture was grown for 48 h at 28° and 240 rpm on a rotary shaker. *C. elegans* spores were inoculated into 2 liters of MNB medium (see below) containing 1% glucose and the culture grown under the same conditions as for *C. echinulata*. Compounds **1** or **3** were dissolved in EtOH (final volumes of EtOH were not more than 2% of the total medium volume) and added to the culture and further incubated for

another 48 h. The composition of the media used for filamentous fungi was: SSBF: glucose 2%, soya bean flour containing 1% fat 0.5%, NaCl 0.5%, KH_2PO_4 0.5%, yeast extract 0.5%, pH 7.0 (20); MNB: glucose 1%, malt extract 2%, nutrient broth 0.8%, pH 6.2. The EtOAc extracts of cell suspensions of separate broth and mycelia fractions were analyzed for metabolites of compounds **1** and **3** by tlc and the individual products purified as described below.

METABOLITE PURIFICATION FROM EXTRACTS OF *C. ECHINULATA*.—After 48 h of biotransformation of isotriptophenolide [**1**] (180 mg, 579 mmol) in 1.8 liters of SSBF medium, the broth and mycelia were separated by filtration through Miracloth, extracted separately with 3 volumes of EtOAc, the extracts were then combined and dried over Na_2SO_4, and evaporated under vacuum. The crude residue was dissolved in $CHCl_3$-MeOH (95:5) and chromatographed on Si gel 60 (230–400 mesh). The product from this separation was examined by tlc (Si gel 60 F_{254}, mobile phase $CHCl_3$-MeOH, 4:1); **2** had an R_f value of 0.44 (data not shown). The above column fraction was further purified by radial chromatography using $CHCl_3$-MeOH (9:1). The yield of **2** from 180 mg of starting material [**1**] was 145 mg (80%) and 17 mg (9%) of unreacted compound **1** was also recovered. Overall recovery was 89%.

PURIFICATION OF METABOLITES FROM EXTRACTS OF *C. ELEGANS*.—Triptophenolide [**3**] (200 mg, 641 mmol) was dissolved in 20.0 ml of EtOH and added directly to 2.0 liters of MNB medium containing a 48-h growing culture of *C. elegans* with pH 4.2 (0.1 mg/ml) which had been inoculated from a spore suspension. The culture was further incubated for 48 h at 240 rpm and 28°. After this period, the cell suspension was filtered through Miracloth in a Buchner funnel and the filtrate and mycelia were extracted separately with EtOAc (3×1 liter). The combined extracts were dried over anhydrous Na_2SO_4. This material was concentrated under vacuum to yield a crude extract (229 mg). This extract was chromatographed over Si gel with hexane-EtOAc (4:1) followed by $CHCl_3$-MeOH (95:5) to afford 2 fractions. Fraction 1 (hexane-EtOAc, 4:1) yielded compounds **5** and **6**. Final purification was performed by radial chromatography with hexane-EtOAc (4:1). Fraction 2 ($CHCl_3$-MeOH, 95:5) yielded compound **7**. The products from this separation were examined by tlc (Si gel 60 F_{254}, mobile phase EtOAc-hexane, 3:2). The R_f values were as follows: [**5**] 0.60, [**6**] 0.21, and [**7**] 0.15 (data not shown). After evaporation of solvent, the percent yields were determined by weighing the vacuum-dried, pure compounds, the mole percent of starting material was then calculated: [**3**] 54 mg (27%), [**5**] 73 mg (35%), [**6**] 26 mg (12%), [**7**] 14 mg (5%). The total recovery from the broth and cells was 79%. The physical properties of the metabolites obtained from the biotransformation of **1** and **3** by *C. echinulata* and *C. elegans* are as follows.

12-Glucosyl isotriptophenolide [**2**].—Obtained as colorless plate-like crystals ($CHCl_3$/MeOH): mp 138–139°; uv (MeOH) λ max (log ε) 276 (0.88), 220 (4.25) nm; ir (Me_2CO) ν max 3486, 2632, 1763, 1702, 1424, 1369, 1233, 1048 cm^{-1}; ^1H nmr ((Me_2CO-d_6, 400 MHz) δ 1.02 (3H, s, Me-20), 1.18 (6H, d, J=7 Hz, Me-16 and 17), 1.69 (1H, ddd, J=7, 12, and 12 Hz, H_A-1), 1.85–1.98 (2H, m, H_2-6), 2.26–2.40 (1H, m, Hβ-2), 2.55 (1H, dd, J=7 and 7 Hz, Hα-2), 2.58–2.63 (1H, m, H_B-1), 2.71 (1H, br d, J=11 Hz, H-5), 2.87–2.94 (2H, m, H-7), 3.42 (1H, septet, J=7 Hz, H-15), 3.48–3.50 (2H, m, H-6′), 3.52 (1H, m, H-2′), 3.59 (1H, dd, J=3 and 11 Hz, H-5′), 3.68 (1H, dd, J=6 and 14 Hz, H-3′), 3.89 (1H, s, $+D_2O$, OH-6′), 3.90 (1H, dd, J=6 and 11 Hz, H-4′), 4.19 (1H, s, $+D_2O$, OH-2′), 4.27 (1H, s, $+D_2O$, OH-3′), 4.42 (1H, s, $+D_2O$, OH-4′), 4.85 (2H, m, H_2-19), 4.93 (1H, d, J=7 Hz, H-1′), 6.94 (1H, s, H-14), 7.18 (1H, s, H-11); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 18.9, 20.7, 22.4, 23.2, 23.4, 26.8, 28.5, 33.2, 37.2, 42.3, 62.8, 71.0, 71.6, 74.8, 77.7, 78.3, 102.7, 112.4, 124.8, 127.5, 129.0, 136.2, 144.6, 154.1, 164.4, 174.2; cims *m/z* 492 $[M+NH_4]^+$, 475 $[M.H]^+$, 459 (base peak), 330, 313, 297, 282, 254, 224, 198, 180, 162; hrcims *m/z* calcd for $C_{26}H_{35}O_8$ $[M+H]^+$ 475.2332, found 475.2337.

Triptoquinone [**5**].—Obtained as yellow crystals (hexane/EtOAc): mp 55–58°; $[\alpha]^{20}D$ +121.6° (c=1.00, $CHCl_3$); uv (MeOH) λ max (log ε) 348 (0.09), 261 (2.06), 227 (3.01), 199 (2.45) nm; ir ($CHCl_3$) ν max 2929, 1756, 1680, 1649, 755 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 1.10 (6H, d, J=7 Hz, Me-16 and 17), 1.14 (3H, s, Me-20), 1.47 (1H, m, H_A-1), 1.69 (1H, m, H_A-6), 1.87 (1H, m, H_B-6), 2.39 (2H, m, H_2-2), 2.48 (1H, ddd, J=4, 8, and 12 Hz, H_A-7), 2.60 (1H, br d, J=10 Hz, H-5), 2.78 (1H, dd, J=8 and 12 Hz, H_B-7), 3.00 (1H, septet, J=7 and 1 Hz, H-15), 3.10 (1H, m, H_B-1), 4.72 (1H, br d, J=16 Hz, H_A-19), 4.81 (1H, br d, J=16 Hz, H_B-19), 6.40 (1H, s, H-12); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 18.3, 18.6, 21.2, 21.2, 24.7, 26.4, 30.7, 30.8, 36.6, 42.3, 70.1, 125.4, 131.6, 142.5, 147.6, 153.4, 161.3, 173.7, 187.1, 187.4; eims *m/z* 326 (M^+) (base peak), 311, 298, 283, 267, 253, 239; hreims *m/z* calcd for $C_{20}H_{22}O_4$, 326.1518, found 326.1523.

5α,14-Dihydroxybutenolide [**6**].—Obtained as fine colorless prisms (hexane/Me_2CO): mp 190–191°; $[\alpha]^{20}D$ −58.8° (c=0.32, CH_3OH); uv (MeOH) λ max (log ε) 282 (3.55), 223 (3.86) nm; ir ($CHCl_3$) ν max 3607 (OH), 3560 (OH), 3020 (aromatic CH), 2973 (CH), 1756 (C=O), 1430, 1240, 1180 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 1.09 (3H, s, Me-20), 1.25, 1.27 (3H each, both d, J=7 Hz, Me-16 and 17), 1.90 (1H, s, $+D_2O$, OH-5), 1.98–2.15 (2H, m, Hα-1 and Hα-6), 2.23 (1H, ddd, J=9, 9, and 14 Hz, Hβ-6), 2.31

(1H, dd, J=6 and 13 Hz, Hβ-1), 2.39 (1H, m, Hβ-2), 2.55 (1H, br d, J=16 Hz, Hα-2), 2.80–3.02 (2H, m, H_2-7), 3.08 (1H, septet, J=7 Hz, H-15), 4.80 (1H, s, OH-14), 4.90 (2H, br d, AB_q, $\Delta\nu$=0.19 ppm, J=17 Hz, H_2-19), 6.91 (1H, d, J=8 Hz, H-11), 7.08 (1H, d, J=8 Hz, H-12); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 18.0, 18.3, 22.5, 22.6, 25.4, 26.2, 26.3, 26.9, 41.3, 69.2 (C-19), 69.8 (C-5), 117.1, 119.7, 124.1, 127.1, 131.4, 140.4, 150.7, 160.9, 173.8; eims *m/z* 328 [M^+], 310, 295, 267, 253 (base peak), 165, 147; hreims *m/z* calcd for $C_{20}H_{24}O_4$ 328.1674; found 328.1675.

14-Glucosyl-triptophenolide [**7**].—Obtained as colorless fine crystals ($CHCl_3$/MeOH): mp 139–141°, uv (MeOH) λ max (log ε) 270 (0.89), 217 (2.66), 195 (0.58) nm; ir (Me_2CO) ν max 3608, 3005, 1713, 1422, 1364, 1223, 1093, 904 cm^{-1}; ^{1}H nmr (Me_2CO-d_6, 400 MHz) δ 1.02 (3H, s, Me-20), 1.13 (3H, d, J=7 Hz, Me-16), 1.17 (3H, d, J=7 Hz, Me-17), 1.68 (1H, ddd, J=7, 12, and 12 Hz, H_A-1), 1.86 (2H, m, H-6), 2.35 (1H, m, Hβ-2), 2.35 (1H, dd, J=6 and 12 Hz, H_B-1), 2.56 (1H, dd, J=6 and 7 Hz, Hα-2), 2.67 (1H, dd, J=2 and 14 Hz, H-5), 3.03–3.11 (1H, dd, J=7 and 17 Hz, H_A-7), 3.17–3.21 (2H, m, H-4′ and H-6′), 3.22–3.32 (1H, dd, J=9 and 17 Hz, H_B-7), 3.43–3.57 (3H, m, H-2′, H-3′, and H-6′), 3.62–3.68 (1H, m, H-5′), 3.65 (1H, s, +D_2O, OH-6′), 3.66 (1H, septet, J=5 Hz, H-15), 4.17 (1H, s, +D_2O, OH-2′), 4.26 (1H, s, +D_2O, OH-3′), 4.60 (1H, s, +D_2O, OH-4′), 4.71 (1H, d, J=7 Hz, H-1′), 4.85 (2H, m, H-19), 7.13 (1H, d, J=8 Hz, H-12), 7.21 (1H, d, J=8 Hz, H-11); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 18.8, 20.3, 22.1, 24.0, 24.1, 26.0, 29.8, 33.5, 37.1, 41.8, 62.9, 71.0, 71.6, 75.6, 77.4, 78.1, 105.8, 110.6, 121.5, 124.5, 129.3, 140.6, 145.1, 153.0, 164.4, 172.5; cims *m/z* 492 [$M+NH_4$]$^+$, 475 [$M+H$]$^+$, 330 (base peak), 313, 297, 197, 180; hrcims *m/z* calcd for $C_{26}H_{35}O_8$ [$M+H$]$^+$ 475.2332, found 475.2339.

ACKNOWLEDGMENTS

The authors are grateful for cooperation and discussions with Professor J.P. Kutney and Dr. M. Miyakoshi of the Department of Chemistry, University of British Columbia. This work was supported by grants to M.M. and J.P.K. from the British Columbia Health Research Foundation and the Natural Sciences and Engineering Research Council of Canada.

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Received 15 June 1994