Antiviral Agents of Plant Origin. II.[1] Antiviral Activity of Scopadulcic Acid B Derivatives

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Scopadulcic acid B derivatives were synthesized and their antiviral activities against herpes simplex virus type 1 (HSV-1) were examined. All the derivatives synthesized showed lower inhibitory activities against HSV-1 than scopadulcic acid B (2). Five compounds, 7, 8, 15, 16, and 18, however, had *in vitro* therapeutic indexes larger than 7 and were considered to merit further investigation.

Keywords scopadulcic acid B; diterpenoid; antiviral activity; cytotoxicity; structure–activity relationship; HSV-1

Previously we isolated cytotoxic diterpenoids named scopadulcic acids A (**1**) and B (**2**) from a Paraguayan crude drug, "Typychá kuratũ" (*Scoparia dulcis* L., Scrophulariaceae).[2] Their structures were elucidated as diterpene acids with a novel skeleton similar to that of aphidicolin (**3**), which is an antibiotic obtained from the mold *Cephalosporium aphidicola* PETCH[3] and has been shown to inhibit deoxyribonucleic acid (DNA) polymerase from rat liver[4] and *Hemicentrotus pulcherrimus*[5] and the replication of herpes simplex virus type 1 (HSV-1).[6,7] *In vitro* examination of the antiviral activity of **1** and **2** against HSV-1 revealed that only scopadulcic acid B inhibited the viral replication.[1] Single-cycle replication experiments indicated that **2** interfered considerably with early events of virus growth. In an *in vivo* assay system using a golden hamster test model, **2** produced a significant delay of the appearance of corneal lesion and prolonged the survival time of the animals.[1]

In order to evaluate further this compound as an antiviral agent, we have prepared various derivatives of **2** and compared their antiviral activities. In this paper we describe the derivatization of scopadulcic acid B and the antiviral activities of the derivatives, and we discuss some structure–activity relationships.

Results and Discussion

Derivatization of Scopadulcic Acid B Reduction of the ketone at the C-13 position of **2** with $NaBH_4$ gave a mixture

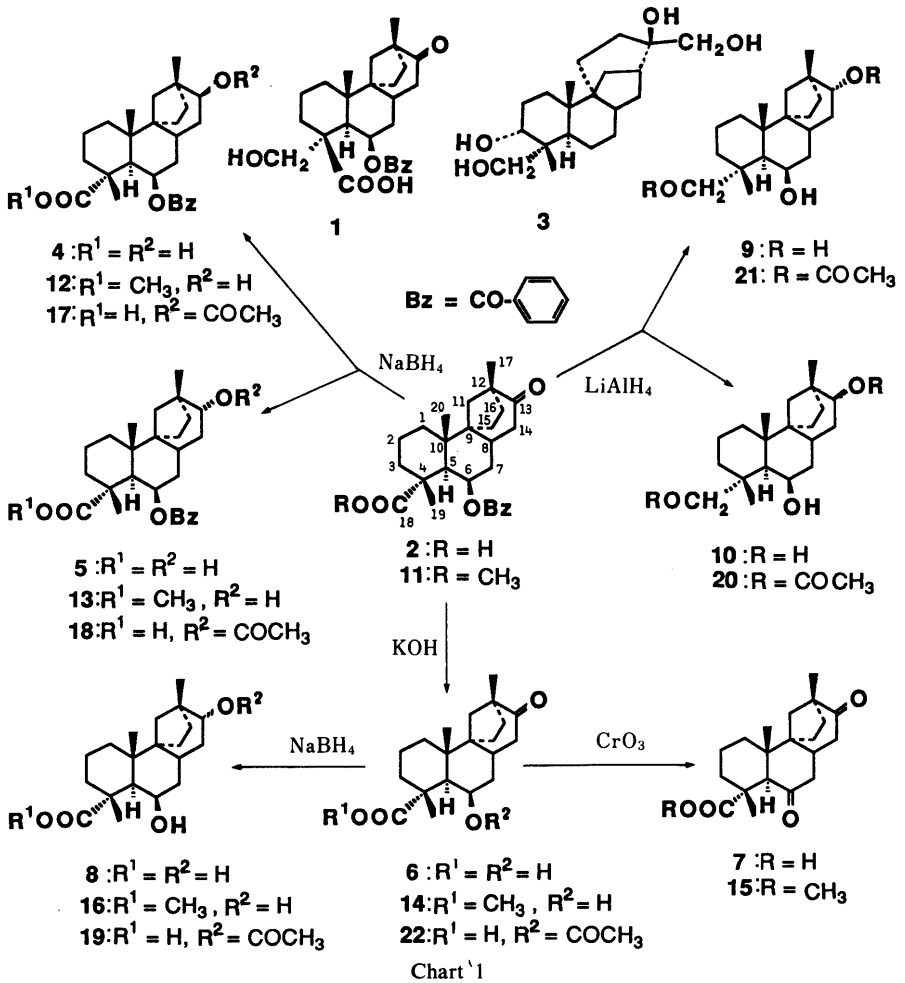

Chart 1

TABLE I. Anti-HSV-1 Activity and Cytotoxicity of Scopadulcic acid B (2) and Its Derivatives

Compound	1	2	3	4	5	6	7	8	9	10	11
ID_{50} [a]	2.2	0.9	210	45.5	27.3	898	199	>898	290	190	17.8
ED_{50} [b]	1.7	0.039	0.24	29.5	13.4	254	22.6	95.2	74	65	6.7
ID_{50}/ED_{50} [c]	1.3	23.1	875	1.5	2.0	3.5	8.8	≧10	3.9	2.9	2.7

Compound	12	13	14	15	16	17	18	19	20	21	22
ID_{50} [a]	8.1	14.8	210	202	226	45	69	228	180	36.9	253
ED_{50} [b]	5.1	7.7	34.5	27.7	31.4	9.8	8.6	146	59.1	13.1	50.5
ID_{50}/ED_{50}	1.6	1.9	6.1	7.3	7.2	4.6	8.0	1.6	3.0	2.8	5.0

a) Dose required to reduce the viability of uninfected HeLa cells by 50% after incubation (μM). *b*) The 50% antiviral effective dose (μM). *c*) *In vitro* therapeutic index. All data represent average values for three separate experiments.

of 13β- and 13α-hydroxyl compounds (**4** and **5**) which were separated by silica gel column chromatography. Hydrolysis of **2** with KOH yielded a debenzoylated product (**6**) which was then transformed to **7** and **8** by Jones oxidation and $NaBH_4$ reduction, respectively. On treatment of **2** with $LiAlH_4$, a mixture of triols (**9** and **10**) was obtained which was also separated by column chromatography. Compounds **2**, **4**—**8** gave the corresponding methyl esters (**11**—**16**) on treatment with diazomethane in ether. Acetylation of **4**, **5**, **8**—**10** with acetic anhydride (Ac_2O) in pyridine gave the corresponding acetates (**17**—**21**). Finally, compound **6** was treated with Ac_2O and 4-dimethylaminopyridine in pyridine to furnish an acetate **22**.

Biological Activities Table I summarize the anti-HSV-1 activities and cytotoxicities of **2** and its derivatives. In this experiment, aphidicolin (**3**) was used as a reference agent for checking the *in vitro* antiviral activity assay system. As shown in Table I, it exerted inhibitory action against HSV-1 with the ID_{50} of 210 μM and the ED_{50} of 0.24 μM. The resulting therapeutic index (the ratio between cytotoxicity and inhibition of virus replication), 875, is in accordance with the value obtained by Bucknall *et al.*[6] All the synthesized derivatives showed lower cytotoxicities than **2** against HeLa 229 cells. When *in vitro* therapeutic indexes were compared, **2** was found to have the highest selective toxicity against HSV-1. The decrease of antiherpetic activity by modification of the carboxyl group at the C-4 position, the benzoyl group at the C-6 position and/or the ketone at the C-13 position suggests that these functional groups might play important roles in manifestation of the antiviral effect.

Recently Nasr *et al.* proposed that unless *in vitro* antiviral activity was separated from cytotoxicity by at least a factor of seven- to eightfold, compounds might not merit additional consideration.[8] On this basis, compounds **2**, **7**, **8**, **15**, **16**, and **18** were considered to be active antiviral compounds and to merit further investigation. The mechanism by which **2** inhibits HSV-1 replication, especially its effect on DNA polymerase derived from the virus, is currently being examined.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Hitachi 260-10 infrared spectrometer. Ultraviolet (UV) spectra were recorded in MeOH on a Hitachi 220S double beam spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a JEOL XL-270 spectrometer and chemical shifts are given in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard. The high-resolution mass spectra (MS) were obtained with a JEOL JMS-D200 mass spectrometer. Specific rotations were taken on a JASCO DIP-140 digital polarimeter.

6β-Benzoyl-12-methyl-9(12)$_a$,9(12)$_b$-dihomo-podocarpane-13β-ol-18-oic Acid (4) and 6β-Benzoyl-12-methyl-9(12)$_a$,9(12)$_b$-dihomo-podocarpane-13α-ol-18-oic Acid (5) A mixture of **2** (226 mg, 0.52 mmol) and $NaBH_4$ (100 mg, 2.64 mmol) was stirred in MeOH (20 ml) for 1 h. After decomposition of excess $NaBH_4$ by adding acetone, the solvent was evaporated off. Water was added to the residue and the mixture was extracted with EtOAc. The concentrated EtOAc extract was chromatographed on a silica gel column with $CHCl_3$. The first fraction eluted with $CHCl_3$ was evaporated to dryness and the residue was recrystallized from $CHCl_3$–MeOH to give **4** (46 mg, 20%) as colorless needles. mp 150—153 °C. $[\alpha]_D$ −59.6° (c=0.4, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1710, 1700, 1600, 1580. UV λ_{max}^{MeOH} nm (log ε): 229 (4.01), 272 (2.98), 280 (2.92). ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, s, 17-CH_3), 1.34 (3H, s, 19-CH_3), 1.56 (3H, s, 20-CH_3), 3.45 (1H, d, J=15 Hz, 13-H), 5.27 (1H, brs, 6-H), 7.44 (2H, t, J=7.3 Hz, ArH), 7.56 (1H, t, J=7.3 Hz, ArH), 8.04 (d, J=7.3 Hz, ArH). MS m/z: 440.2594 (M^+, $C_{27}H_{36}O_5$ requires 440.2561).

The second fraction eluted with $CHCl_3$ was evaporated to dryness and the residue was recrystallized from $CHCl_3$ to afford **5** (148 mg, 65%) as colorless needles. mp 201—203 °C. $[\alpha]_D$ −70.0° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1710, 1700, 1600. UV λ_{max}^{MeOH} nm (log ε): 229 (4.15), 272 (3.07), 280 (2.99). ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, s, 17-CH_3), 1.34 (3H, s, 19-CH_3), 1.51 (3H, s, 20-CH_3), 3.40 (1H, dd, J=10.0, 6.0 Hz, 13-H), 5.26 (1H, d, J=1.5 Hz, 6-H), 7.45 (1H, t, J=7.3 Hz, ArH), 7.57 (1H, t, J=7.3 Hz, ArH), 8.04 (2H, d, J=7.3 Hz, ArH). MS m/z: 440.2525 (M^+, $C_{27}H_{36}O_5$ requires 440.2561).

12-Methyl-9(12)$_a$,9(12)$_b$-dihomo-podocarpane-6β-ol-13-one-18-oic Acid (6) A solution of **2** (100 mg, 0.23 mmol) in dimethyl sulfoxide (DMSO) (0.2 ml) was added to a saturated solution of KOH in MeOH (1 ml) and the mixture was heated at 120 °C for 15 h in a sealed tube, then allowed to cool. An equal volume of water was added and the whole was acidified with 1 N HCl. The reaction mixture was extracted with EtOAc and the EtOAc layer was concentrated *in vacuo*. The residue was chromatographed on a silica gel column with $CHCl_3$ to give benzoic acid (12 mg) and **6** as colorless needles (34 mg, 45%). mp 224—228 °C. $[\alpha]_D$ +5.5° (c=0.4, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500, 3420, 3250, 1710, 1690. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (3H, s, 17-CH_3), 1.36 (3H, s, 19-CH_3), 1.58 (3H, s, 20-CH_3), 4.02 (1H, d, J=2.0 Hz, 6-H). MS m/z: 334.2156 (M^+, $C_{20}H_{30}O_4$ requires 334.2142).

12-Methyl-9(12)$_a$,9(12)$_b$-dihomo-podocarpane-6,13-dione-18-oic Acid (7) An ice-cold solution of **6** (53 mg, 0.16 mmol) in acetone (4 ml) was treated with Jones reagent and worked up as usual. The reaction mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with saturated NaCl, dried, and concentrated *in vacuo*. The residue was chromatographed on a silica gel column using $CHCl_3$ as the eluent to afford **7** as a colorless amorphous powder (47 mg, 89%). $[\alpha]_D$ +54.0° (c=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500, 1700. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, s, 17-CH_3), 1.13 (3H, s, 19-CH_3), 1.46 (3H, s, 20-CH_3). MS m/z: 332.2009 (M^+, $C_{20}H_{28}O_4$ requires 332.1986).

12-Methyl-9(12)$_a$,9(12)$_b$-dihomo-podocarpane-6β,13α-diol-18-oic Acid (8) A solution of **6** (50 mg, 0.15 mmol) in MeOH (5 ml) was treated with $NaBH_4$ (20 mg, 0.53 mmol) and the reaction mixture was stirred for 2 h, then worked up as previously described to give **8** as colorless needles (34 mg, 68%). mp >300 °C. $[\alpha]_D$ +37.0° (c=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3480, 3360, 1700. ^{1}H-NMR ($CDCl_3$) δ: 1.07 (3H, s, 17-CH_3), 1.32 (3H, s,

TABLE II Yields, Physical Constants and Analytical Data for the Methylesters (**11**—**16**)

Compd. No.	Yield (%)	mp (°C)	Formula	NMR ($CDCl_3$)	Analysis (%) or high MS Calcd (Found)
11	31	190—191[a]	$C_{28}H_{36}O_5$	1.09 (3H, s), 1.35 (3H, s), 1.54 (3H, s) 3.63 (3H, s), 5.20 (1H, d, J= 2.0 Hz), 7.46 (2H, t, J=7.3 Hz), 7.56 (1H, t, J=7.3 Hz), 8.02 (2H, d, J=7.3 Hz)	C, 74.30; H, 8.02 (C, 74.11; H, 7.92)
12	87	—	$C_{28}H_{38}O_5$	1.03 (3H, s), 1.34 (3H, s), 1.55 (3H, s), 3.44 (1H, br s), 3.66 (3H, s), 5.14 (1H, br d, J=2.4 Hz), 7.45 (2H, t, J=7.3 Hz), 7.56 (1H, t, J=7.3 Hz), 8.04 (2H, d, J=7.3 Hz)	454.2717 (454.2731)
13	92	—	$C_{28}H_{38}O_5$	1.03 (3H, s), 1.34 (3H, s), 1.51 (3H, s), 3.40 (1H, dd, J=10.3, 5.9 Hz), 3.66 (3H, s), 5.14 (1H, br d, J=2.4 Hz), 7.46 (2H, t, J=7.3 Hz), 7.57 (1H, t, J=7.3 Hz), 8.04 (2H, d, J=7.3 Hz)	454.2717 (454.2734)
14	53	—	$C_{21}H_{32}O_4$	1.08 (3H, s), 1.36 (3H, s), 1.57 (3H, s), 3.67 (3H, s), 3.88 (1H, br s)	348.2299 (348.2270)
15	91	131—133[a]	$C_{21}H_{30}O_4$	1.01 (3H, s), 1.12 (3H, s), 1.45 (3H, s), 3.64 (3H, s)	346.2144 (346.2152)
16	96	186—188[a]	$C_{21}H_{34}O_4$	1.02 (3H, s), 1.56 (6H, s), 3.45 (1H, m)	C, 71.96; H, 9.78 (C, 71.57; H, 9.38)

a) Recrystallized from $CHCl_3$–MeOH.

TABLE III. Yields, Physical Constants, and Analytical Data for the Acetates (**17**—**22**)

Compd No.	Yield (%)	mp (°C)	Formula	NMR ($CDCl_3$)	Analysis (%) or high MS Calcd (Found)
17	69	—	$C_{29}H_{38}O_6$	0.94 (3H, s), 1.37 (3H, s), 1.56 (3H, s), 2.03 (3H, s), 4.63 (1H, t, J= 1.5 Hz), 5.27 (1H, d, J=2.0 Hz), 7.47 (2H, t, J=7.3 Hz), 7.58 (1H, t, J=7.3 Hz), 8.07 (2H, d, J=7.3 Hz)	482.2666 (482.2588)
18	87	—	$C_{29}H_{38}O_6$	0.95 (3H, s), 1.34 (3H, s), 1.52 (3H, s), 2.02 (3H, s), 4.62 (1H, dd, J= 10.3, 5.9 Hz), 5.28 (1H, d, J=2.0 Hz), 7.46 (2H, t, J=7.3 Hz), 7.57 (1H, t, J=7.3 Hz), 8.03 (2H, d, J=7.3 Hz)	482.2666 (482.2602)
19	53	—	$C_{22}H_{34}H_5$	0.94 (3H, s), 1.33 (3H, s), 1.57 (3H, s), 2.03 (3H, s), 3.96 (1H, t, J= 2.2 Hz), 4.65 (1H, dd, J=10.6, 5.9 Hz)	378.2404 (378.2411)
20	93	—	$C_{24}H_{38}O_5$	0.93 (3H, s), 1.28 (3H, s), 1.37 (3H, s), 2.05 and 2.07 (each 3H, s), 3.68 and 4.00 (each 1H, d, J=11.2 Hz), 4.19 (1H, br s), 4.61 (1H, t, J=2.0 Hz)	C, 70.90; H, 9.42 (C, 70.52; H, 9.14)
21	93	154—157[a]	$C_{24}H_{38}O_5$	0.93 (3H, s), 1.28 (3H, s), 1.37 (3H, s), 2.05 and 2.07 (each 3H, s), 3.68 and 4.00 (each 1H, d, J=11.2 Hz), 4.19 (1H, br s), 4.65 (1H, dd, J=10.7, 6.1 Hz)	C, 70.90; H, 9.42 (C, 70.93; H, 9.20)
22	73	—	$C_{22}H_{32}O_5$	1.09 (3H, s), 1.34 (6H, s), 2.08 (3H, s), 5.02 (1H, br d, J=2.0 Hz)	376.2248 (376.2203)

a) Recrystallized from $CHCl_3$–MeOH.

19-CH_3), 1.37 (3H, s, 20-CH_3), 3.44 (1H, dd, J=10.7, 6.4 Hz, 13-H), 3.95 (1H, d, J=1.5 Hz, 6-H). MS m/z: 336.2280 (M^+, $C_{20}H_{32}O_4$ requires 336.2299).

12-Methyl-9(12)$_a$,9(12)$_b$-dihomo-6β,13α,18-podocarpanetriol(9) and 12-Methyl-9(12)$_a$,9(12)$_b$-dihomo-6β,13β,18-podocarpanetriol (10) A solution of **2** (101 mg, 0.23 mmol) in dry ether (10 ml) was treated with $LiAlH_4$ (100 mg, 2.64 mmol). After stirring of this solution for 1 h at room temperature, EtOAc was added and the reaction mixture was then washed with 10% HCl and water successively. The EtOAc layer was concentrated to give a viscous oil, which was chromatographed on a silica gel column using $CHCl_3$ as the eluting solvent. The first eluate was evaporated and the residue was recrystallized from $CHCl_3$–MeOH to give **9** as colorless needles (35 mg, 47%). mp 221—223 °C. $[\alpha]_D$ −31.5° (c=0.4, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3430. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, s, 17-CH_3), 1.18 (3H, s, 19-CH_3), 1.32 (3H, s, 20-CH_3), 3.17 and 3.52 (1H, each d, J=11.0 Hz, 18-H), 3.43 (1H, dd, J=10.5, 5.9 Hz, 13-H), 4.24 (1H, dd, J=5.1, 2.2 Hz, 6-H). MS m/z: 322.2439 (M^+, $C_{20}H_{34}O_3$ requires 322.2506).

The second eluate was evaporated to yield **10** as a colorless amorphous powder (20 mg, 27%). $[\alpha]_D$ −11.9° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3430. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, s, 17-CH_3), 1.19 (3H, s, 19-CH_3), 1.37 (3H, s, 20-CH_3), 3.17 and 3.54 (1H, each d, J=11.0 Hz, 18-H), 3.44 (1H, d, J=3.7 Hz, 13-H), 4.24 (1H, dd, J=5.1, 2.6 Hz, 6-H). MS m/z: 322.2516 (M^+, $C_{20}H_{34}O_3$) requires 322.2506).

Preparation of Methylesters (11—16) A solution of **2** (100 mg, 0.23 mmol) in ether was treated with CH_2N_2 for 15 h. After evaporation to dryness, a crystalline material was obtained, which on recrystallization from $CHCl_3$–MeOH furnished **11** as colorless needles (30 mg, 31%). Compounds **12**—**16** were obtained from **4**—**8**, respectively, in the same manner as described for **11**. The yields, melting points, NMR spectral data, and high MS data or elemental analysis data are given in Table II.

Preparation of Acetates (17—22) A solution of **4** (25 mg, 57 μmol) in pyridine (0.5 ml) was treated with Ac_2O (0.5 ml) at room temperature for 15 h. Work-up of the reaction mixture in the usual manner gave a product which was purified by silica gel column chromatography using $CHCl_3$ as the eluent to furnish **17** as a colorless amorphous powder (19 mg, 69%). Compounds **18**—**21** were obtained from **5**, **8**, **10**, and **9**, respectively, in the same manner as described for **17**. Compound **22** was prepared from **6** by reaction with Ac_2O and 4-dimethylaminopyridine in pyridine at room temperature for 15 h. The yields, melting point, NMR spectral data, and high MS data or elemental analysis data are summarized in Table III.

Biological Evaluation (1) Cell Culture and Viruses HeLa 229 cells were cultured in Eagle's minimal essential medium (MEM) containing 5% fetal calf serum (FCS) for use in growth assay of HSV-1 or plaque assay. Stock suspensions of HSV-1 strain HF were prepared from infected HeLa cells.

(2) Preparation of Drug Samples All compounds including aphidicolin were dissolved in DMSO and then dispersed in the culture medium to give a final DMSO concentration of less than 0.5% (v/v). At these concentrations, DMSO did not affect the cytotoxicity or antiviral activity.

(3) Determination of 50% Inhibitory Dose (ID_{50}) for Cell Growth HeLa cells precultured for 24 h at 37 °C in MEM plus 5% FCS were allowed to grow for an additional 24 h in the presence of increasing amounts of the compound. Cell viability was determined by the trypan blue exclusion method.[9] The number of cells was plotted against drug concentration on a

semilogarithmic scale. The ID_{50} is the concentration of drug that inhibited cell growth by 50% as compared with the no-drug control.

(4) Assay for Anti-HSV-1 Activity HeLa cells infected with HSV-1 at a multiplicity of infection of 0.5 were incubated at 34 °C in the medium (MEM plus 2% FCS) containing various amounts of the test compound and harvested after 24 h. Virus yields were determined by plaque assay. The ED_{50} was determined as the least drug concentration which reduced plaque numbers by 50% in the drug-treated culture compared to untreated cultures.

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