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NEW STEROID ACIDS FROM *ANTRODIA CINNAMOMEA*, A FUNGAL PARASITE OF *CINNAMOMUM MICRANTHUM*

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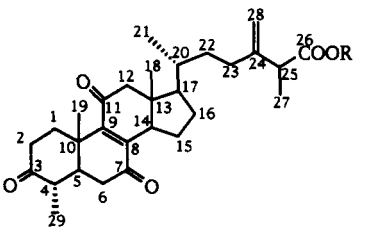
ABSTRACT.—Three new steroids, zhankuic acids A [**1**], B [**2**], and C [**3**], were isolated from the fruiting bodies of *Antrodia cinnamomea* by bioassay-guided fractionation. The structures of these compounds were elucidated by chemical reactions and detailed analysis of their ^{1}H- and ^{13}C-nmr spectra. Biological studies revealed that **1** exhibited cytotoxic activity against P-388 murine leukemia cells and **2** showed weak anticholinergic and antiserotonergic activities.

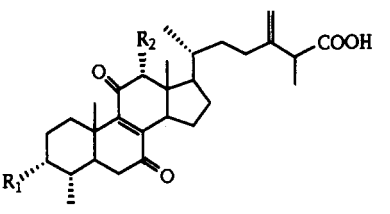
Antrodia cinnamomea Chang & Chou, sp. nov. (Polyporaceae, "Zhan Ku," "Zhan Chih"), which is a microorganism parasitic to the inner wall of the heart wood of the Taiwanese evergreen tree *Cinnamomum micranthum* (Hayata) Hayata, has been utilized in traditional Chinese medicine for the treatment of food and drug intoxications, diarrhea, abdominal pain, hypertension, skin itching, and liver cancer (1). Due to these interesting biological activities and the potential clinical applications, it was considered of interest to explore the active constituents of this fungus. Preliminary pharmacological testing on a guinea pig ileum preparation revealed that a crude EtOH extract of *A. cinnamomea* possessed weak anticholinergic and antiserotonergic activities (ED_{50} values of 100 μg/ml). Also, biological evaluation of a crude $CHCl_3$/MeOH extract from another collection of this species exhibited significant cytotoxicity against P-388 murine leukemia cells (IC_{50} 4.1 μg/ml). Bioassay-guided fractionation of the EtOH extract of *A. cinnamomea* led to the isolation of three new steroidal acids, which were accorded the trivial names zhankuic acids A–C, and assigned as 4α-methylergosta-8,24(28)-dien-3,7,11-trione-26-oic acid [**1**], 3α-hydroxy-4α-methylergosta-8,24(28)-dien-7,11-dione-26-oic acid [**2**], and 3α,12α-dihydroxy-4α-methylergosta-8,24(28)-dien-7,11-dione-26-oic acid [**3**], respectively, based on chemical and spectral analyses.

RESULTS AND DISCUSSION

An EtOH extract of the dried fruiting bodies of *A. cinnamomea* was fractionated by solvent partitioning and chromatographic separation. Bioassay-guided cc separation revealed that the lipophilic fraction possessed significant anticholinergic, antiserotonergic, and cytotoxic activities. Further separation of the lipophilic layer by a combination of



1 R=H
4 R=Me



2 R_1=OH, R_2=H
3 R_1=R_2=OH
5 R_1=OAc, R_2=H
6 R_1=R_2=OAc

Sephadex LH-20 cc, Si gel cc, and prep. tlc furnished zhankuic acids A [**1**], B [**2**], and C [**3**] in yields of 1%, 0.02%, and 0.35%, respectively.

Zhankuic acid A [**1**], $C_{29}H_{40}O_5$, was obtained as pale-yellow needles. The uv absorption maximum (266 nm) and an ir band (1680 cm^{-1}) suggested the presence of a conjugated ene-dione, and the ir spectrum also indicated terminal methylene and unconjugated ketone functions (902 and 1708 cm^{-1}). Methylation of **1** yielded **4**, which exhibited a M^+ at *m/z* 482 in the eims, suggesting the presence of a free carboxylic unit in **1**. The ^{1}H-nmr spectrum of **1** (Table 1) showed three methyl doublets (H-21, H-27, H-29), two methyl singlets (H-18, H-19), and two terminal olefinic methylene protons (H-28, δ 4.88, and 4.94). The ^{13}C-nmr spectrum displayed three carbonyl singlets, four olefinic carbons and one carboxyl singlet (Table 2). Other signals including five methyl, eight methylene, six methine, and two sp^3 quaternary carbons were also observed in the ^{13}C-nmr spectrum. The carbon signals of the conjugated ene-dione moiety of **1** were similar to those of the 7,11-dione-8-ene system in methyl ganoderates isolated from *Ganoderma lucidum* (2–5). This finding suggested that compound **1** contains an androsta-8-ene-7,11-dione ring skeleton and a side-chain probably similar to those of the ganoderic acids. The relationships between these protons in **1** were further proved by COSY nmr experiments. The H-1 methylene (δ 1.40, δ 3.03) correlated with the neighboring H-2 methylene (δ 2.48, δ 2.50) forming an AMX_2 pattern. The H-4 signal showed correlations not only with H_3-29 but also with H-5, which was coupled to H_2-6. Correlations between H-14, H_2-15, H_2-16, and H-17 were also observed. In the side-chain, H_2-22 showed correlations with H_2-23, and one of the latter protons exhibited a

TABLE 1. ^{1}H-Nmr Spectral Data (300 MHz, $CDCl_3$) for Compounds **1–3**.[a]

Proton(s)	Compound		
	1	**2**	**3**
1α	1.40 m	1.40 m	1.35 m
1β	3.04 ddd (2.7, 6.6, 13.4)	2.50 m	2.22 m
2	2.48 m, 2.50 m	1.70 m, 1.80 m	1.75 m
3		3.78 br s	3.76 br s
4	2.42 m	1.72 m	1.70 m
5	1.88 td (12.7, 4.3)	2.13 td (10.8, 4.3)	2.03 m
6α	2.43 m	2.39 dd (15, 4.3)	2.40 m
6β	2.46 m	2.23 t (15)	2.22 m
12α	2.37 br d (14)	2.39 br d (14)	
12β	2.91 d (14)	2.87 d (14)	4.02 s
14	2.63 dd (7.3, 11.9)	2.60 dd (11.8, 7.3)	2.98 dd (12.2, 7.4)
15	1.40 m, 2.45 m	1.40 m, 2.50 m	1.42 m, 2.50 m
16	1.20 m, 2.00 m	1.25 m, 1.98 m	1.25 m, 1.95 m
17	1.40 m	1.40 m	1.85 m
18	0.66 s	0.65 s	0.61 s
19	1.50 s	1.29 s	1.26 s
20	1.40 m	1.40 m	1.42 m
21	0.90 d (5.4)	0.91 d (5.3)	0.92 d (6.3)
22	1.18 m, 1.55 m	1.18 m, 1.56 m	1.15 m, 1.55 m
23	1.95 m, 2.18 m	1.95 m, 2.16 m	1.87 m, 2.15 m
25	3.11 q (7.0)	3.13 q (7.0)	3.16 q (7.0)
27	1.26 d (7.0)	1.28 d (7.0)	1.28 d (7.0)
28b	4.94 br s	4.96 br s	4.95 br s
28a	4.88 d (2.6)	4.94 br s	4.90 br s
29	1.01 d (6.5)	0.94 d (6.8)	0.91 d (6.5)

[a]δ in ppm (*J* in Hz); TMS as internal standard.

TABLE 2. ^{13}C-Nmr Spectral Data (75 MHz)for Compounds **1–3**.[a]

Carbon	Compound		
	1	**2**	**3**
1	34.7 t	27.9 t	27.9 t
2	37.5 t	29.2 t	29.0 t
3	210.8 s	70.4 d	70.4 d
4	43.9 d	34.7 d	34.6 d
5	48.8 d	41.2 d	40.8 d
6	38.9 t	38.1 t	38.4 t
7	200.8 s	202.2 s	201.7 s
8	145.5 s	144.7 s	144.8 s
9	151.9 s	153.8 s	152.3 s
10	38.3 s	38.8 s	38.4 s
11	202.6 s	202.8 s	202.7 s
12	57.3 t	57.6 t	80.8 d
13	47.1 s	47.4 s	49.7 s
14	49.2 d	49.6 d	42.0 d
15	24.8 t	25.0 t	24.0 t
16	27.8 t	27.9 t	26.9 t
17	53.9 d	54.1 d	45.7 d
18	11.9 q	12.0 q	11.5 q
19	16.2 q	16.2 q	16.1 q
20	35.6 d	35.7 d	35.3 d
21	18.5 q	18.6 q	17.9 q
22	33.8 t	34.0 t	34.0 t
23	31.3 t	31.5 t	30.8 t
24	148.0 s	148.3 s	148.2 s
25	45.6 d	45.5 d	45.7 d
26	180.1 s	178.8 s	179.0 s
27	16.1 q	16.2 q	16.1 q
28	111.4 t	111.5 t	111.5 t
29	11.4 q	15.7 q	15.6 q

[a]Multiplicities determined by DEPT; measured in $CDCl_3$.

long-range correlation with H-28. COLOC experiments were performed to incorporate these fragments into the structure of **1** (Figure 1). The protons of Me-19 (δ 1.50) coupled with C-1, C-5, C-9, and C-10, and H_3-29 (δ 1.10) coupled with C-4 and C-5. This allowed the establishment of rings A and B in **1**. The two carbonyl carbons, C-7 (δ 200.8) and C-11 (δ 202.6), showed correlations with the H_2-6 and H_2-12 methylene protons, respectively. Similarly, the correlations of H_3-18 and C-12, C-13, C-14, and C-17, and the correlations of H_3-21 and C-17, C-20, and C-22 established rings C and D and also confirmed the location of the side-chain moiety at C-17. Finally, the terminal methylene protons (H-28) were found to couple with C-23 and C-25, which in turn showed a correlation with H_3-27 (δ 1.26).

Additional proof for the assignment of zhankuic acid A as **1** was obtained from eims fragmentation studies on **1**. The fragment ion, *m/z* 354, was derived from a McLafferty-type cleavage between C-22 and C-23, and the fragment ion at *m/z* 311 from the cleavage between C-17 and C-20, followed by the loss of two protons, and confirmed the expected basic structure of **1** (6,7). NOe difference studies revealed that the configurations of H-5, Me-21, and Me-29 were α-oriented. Irradiation of the methyl (H-29) at δ 1.01 caused enhancement of the H-5 (δ 1.88) and H-6 (δ 2.46) signals by 2.2% and 5.1%, respectively. The signals of H-12β (δ 2.91) and H-21 (δ 0.90) were enhanced (each 1%)

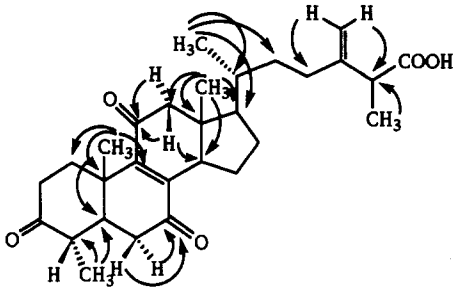


FIGURE 1. COLOC correlations of **1**.

by irradiating the methyl singlet H-18 at 0.66 ppm. Accordingly, the chemical shifts and coupling constants of the protons in the side-chain of **5** were identical to those of methyl polyporenate isolated by Bryce *et al.* (8). The position of the methyl signal in ring A of **1** compared with that of the oxidative product of fusidic acid is consistent with the α-configuration of Me-29 (9,10). In the course of our investigation, we routinely observed equal duplicate signals for part of the ^{13}C-nmr bands in the side-chain of zhankuic acid A [**1**]. Although we were unable to further resolve **1**, it seems likely that compound **1** is present as a mixture, epimeric at C-25.

Zhankuic acid B [**2**], $C_{29}H_{42}O_5$, showed ms, ir, and uv data similar to those of **1**. The presence of three methyl doublets, two methyl singlets, and two olefinic methylene protons in the ^{1}H-nmr spectrum of **2** clearly suggested that this compound was an analogue of **1**. The ^{13}C-nmr spectrum of **2** was superimposalbe upon that of **1** except for the signal of C-3 (δ 70.4), indicating that C-3 was hydroxylated in **2**. Acetylation of **2** yielded the monoacetate [**5**], which exhibited an H-3 signal at δ 4.94 (downfield shift 1.16 ppm) and an acetyl resonance at δ 2.01 in the ^{1}H-nmr spectrum. The configuration of C-3 was determined by comparing the chemical shifts and coupling patterns of H-3 and H-29 with those of derivatives of fusidic acid, as mentioned above. Chemical correlation by oxidation of compound **2** with CrO_3 in pyridine provided a product identical to zhankuic acid A [**1**].

Zhankuic acid C [**3**], $C_{29}H_{42}O_6$, had a uv absorption maximum (270 nm) and ir bands (1711, 1674, 901 cm^{-1}) resembling those of **1** and **2**, suggesting that it was a close analogue. The ^{1}H- and ^{13}C-nmr spectra of **3** exhibited signals similar to those of **2** except for the additional methine proton and the hydroxylated carbon (δ 4.02 and δ 80.9) in **3**. As expected, it gave a diacetate [**6**], for which the second methine proton resonated at δ 5.69 in the ^{1}H-nmr spectrum. Comparison of the ^{1}H-^{13}C correlation nmr spectrum of **3** with that of **2** suggested that the additional hydroxyl was located at the C-12 position. The β-configuration of H-12 was determined by the observation of a γ-effect on C-14 and C-17 (upfield shifts $\Delta\delta = -7.6$ and -8.4, respectively) (2). The signals of C-12 and C-13 were shifted downfield (27.6 and 6.5 ppm) and the signal of C-18 was shifted upfield (-5.4 ppm) in **3**, in comparison with the corresponding signals of **2**. Irradiation of the Me-29 signal caused peak enhancements of H-6α, CH_3CO-3, H-5, and H-4 (6.2%, 2%, 5%, and 7%, respectively), indicating that the C-4 methyl substituents in **3** and **2** were α-oriented. The structural assignment was confirmed by COLOC experiments, in a similar manner as for **1**.

Compounds **1–3** possess a new lanosteroid skeleton which is different from that of the constituents of the fungus *G. lucidum* (11). Characteristic structural features worth noting are the terminal methylene group at C-24, the terminal carboxyl group at C-26, an oxidized 8-en-7,11-dione, and, especially, the lack of methyl groups at C-4 and C-14.

Pharmacological studies (Table 3) revealed that **2** showed anticholinergic and antiserotonergic activities as tested on the guinea pig ileum preparation at 10 μg/ml. Compounds **1** and **3** exhibited no activity at the same concentration. However, compounds **1** and **3** exhibited *in vitro* cytotoxicity against P-388 murine lymphocytic leukemia cells, with IC_{50} values of 1.8 and 5.4 μg/ml, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Fisher-Johns apparatus and are reported uncorrected. Optical rotations were measured on a Jasco DIP-360 polarimeter. Uv and ir spectra were taken on a Hitachi 150–120 uv and a Jasco A-100 ir spectrophotometer, respectively. Eims and hreims were obtained on MAT 112S-JMS D300 and JEOL JMS-HX 110 spectrometers, respectively. ^{1}H-, ^{13}C-, DEPT, COSY, COLOC, and nOe nmr spectra were recorded on a Bruker AM 300 spectrometer using TMS as internal standard.

PLANT MATERIAL.—The fruiting bodies of *Antrodia cinnamomea* were purchased from a medicinal plant dealer in Taipei, Taiwan, 1987. This perennial fungus appears to be planar or bell-like and has a strong camphor-like smell. The orange inner layer of the fruiting body is distributed with very dense and small pores around 4–5 units per mm^2. The colorless ball-shape spores are 5–6 μm long and 4–5 μm wide. A voucher specimen has been preserved at the Institue of Marine Resources, National Sun Yat-sen University.

EXTRACTION AND ISOLATION.—The dried fungus (245 g) was ground to a powder and extracted with EtOH (1.5 liter×3). The concentrated extracts were partitioned between EtOAc and H_2O. The EtOAc fraction (71 g, anticholinergic test ED_{50} 50 μg/ml) was chromatographed over a Sephadex LH-20 (500 g) column (50×10 cm i.d.) using EtOH (6 liters) as eluent to yield four fractions. The third fraction (19 g, ED_{50} 25 μg/ml) was chromatographed on a Si gel (450 g) column eluted with $CHCl_3$ and increasing concentrations of MeOH (0.5%, 1%, 2%, 3%, 5%, 10%, 20%, 500 ml each) to provide 14 fractions. Fraction 2 was purified over Si gel (160 g) to give a pale yellow residue, which yielded zhankuic acid A (**1**, 900 mg) after recrystallization. Zhankuic acid B (**2**, 20 mg) was obtained from fraction 4 by prep. tlc (Si gel, toluene-EtOAc-HOAc, 10:1:0.5). Zhankuic acid C (**3**, 300 mg) was obtained directly from the recrystallization of fraction 7.

Zhankuic acid A [**1**].—Pale yellow needles, mp 136–138°; $[\alpha]^{25}$D +77.6° (c=1.47, $CHCl_3$); uv (MeOH) λ max (log ε) 266 (3.80) nm; ir ν max (KBr) 3440, 2942, 2882, 1708, 1680, 1462, 1416, 1384, 1272, 1236, 1108, 1026, 902, 650 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2; hreims *m/z* 468.2872 ($C_{29}H_{40}O_5$, calcd 468.2876); eims *m/z* 468 (M^+, 56), 450 (9), 424 (78), 354 (64), 339 (33), 326 (22), 313 (18), 311 (63), 297 (11), 286 (42), 271 (22), 260 (30), 246 (10), 231 (8), 220 (23), 215 (7), 201 (6), 189 (8), 175 (9), 161 (9), 149 (9), 135 (14), 123 (20), 121 (17) 109 (38), 95 (100), 82 (13), 68 (30).

Zhankuic acid A methyl ester [**4**].—A solution of **1** (100 mg) was treated overnight with an excess amount of CH_2N_2 in Et_2O. The reaction mixture was concentrated *in vacuo* to give a monomethyl ester [**4**]: Uv (MeOH) λ max (log ε) 266 (3.70) nm; ir (KBr) ν max 3379, 2980, 2945, 2883, 1740, 1709, 1678, 1458, 1433, 1417, 1374, 1193, 1171, 1097, 1079, 900 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.40 (1H, m, H-1α), 3.04 (1H, ddd, J=13.4, 6.7, and 2.9 Hz, H-1β), 2.45 (1H, m, H-2), 2.50 (1H, m, H-2), 2.42 (1H, m, H-4), 1.88 (1H, td, J=12.4 and 4.1 Hz, H-5), 2.40 (1H, m, H-6α), 2.50 (1H, m, H-6β), 2.37 (1H, br d, J=14 Hz, H-12α),

TABLE 3. Biological Activities of Zhankuic Acids A [**1**], B [**2**], and C [**3**].

Assay	Compound		
	1	**2**	**3**
P-388 (leukemia)[a]	1.8	NT[c]	5.4
Anticholinergic test[b]	±[c]	±[c]	−[c]
Antiserotonergic test[b]	±[c]	±[c]	−[c]

[a]The concentration (μg/ml) of compound which inhibits 50% (IC_{50}) of the growth of the murine tumor cell line, P-388 (murine lymphocytic leukemia) after 72 h drug exposure.

[b]Guinea pig ileum preparations were employed in these tests. The test compound was added to the preparation to give a final concentration of 10 μg/ml.

[c]"+" positive; "−" negative; "±" amplitude between positive and negative (see Experimental section); "NT" not tested.

2.92 (1H, d, J=14 Hz, H-12β), 2.64 (1H, dd, J=11.8 and 7.3 Hz, H-14), 1.4 (2H, m, H_2-15), 1.2 (2H, m, H_2-16), 1.4 (1H, m, H-17), 0.68 (3H, s, H_3-18), 1.51 (3H, s, H_3-19), 1.40 (1H, m, H-20), 0.92 (3H, d, J=5.6 Hz, H_3-21), 1.18 (1H, m, H-22), 1.63 (1H, m, H-22), 1.94 (1H, m, H-23), 2.10 (1H, m, H-23), 3.11 (1H, q, J=7.0 Hz, H-25), 1.26 (3H, d, J=7.0 Hz, H_3-27), 4.85 (1H, d, J=3.3 Hz, H-28a), 4.90 (1H, d, J=4.1 Hz, H-28b), 1.03 (3H, d, J=6.6 Hz, H_3-29), 3.66 (3H, s, COOMe); ^{13}C nmr ($CDCl_3$) δ 34.7 (t, C-1), 37.4 (t, C-2), 210.3 (s, C-3), 43.9 (d, C-4), 48.9 (d, C-5), 38.9 (t, C-6), 200.5 (s, C-7), 145.4 (s, C-8), 151.9 (s, C-9), 38.3 (s, C-10), 202.3 (s, C-11), 57.3 (t, C-12), 47.1 (s, C-13), 49.3 (d, C-14), 24.8 (t, C-15), 27.7 (t, C-16), 54.1 (d, C-17), 11.9 (q, C-18), 16.3 (q, C-19), 35.6 (d, C-20), 18.5 (q, C-21), 33.9 (t, C-22), 31.2 (t, C-23), 148.4 (s, C-24), 45.7 (d, C-25), 174.8 (s, C-26), 16.3 (q, C-27), 110.9 (t, C-28), 11.4 (q, C-29), 51.7 ($COOCH_3$); eims m/z 482 (M^+, 66), 450 (11), 422 (9), 354 (34), 339 (18), 326 (12), 313 (10), 311 (100), 297 (10), 286 (30), 284 (32), 273 (16), 271 (16), 260 (25), 246 (8), 231 (7), 220 (25), 215 (7), 203 (6), 189 (10), 173 (10).

Zhankuic acid B [**2**].—Pale-yellow needles: mp 188–191°; $[\alpha]^{25}$D +44.4° (c=0.26, $CHCl_3$); uv (MeOH) λ max (log ε) 261 (3.7) nm; ir (KBr) ν max 3436, 2941, 2929, 1708, 1678, 1459, 1381, 1233, 992, 901 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2; hreims m/z 470.3047 ($C_{29}H_{42}O_5$, calcd 470.3032); eims m/z [M]$^+$ 470 (100), 452 (14), 426 (16), 356 (16), 342 (4), 327 (11), 315 (7), 313 (11), 297 (5), 288 (15), 275 (6), 261 (9), 248 (5), 233 (4), 222 (36), 215 (3), 201 (3), 189 (2), 175 (5), 109 (5), 95 (6).

Zhankuic acid B acetate [**5**].—Acetylation (Ac_2O-pyridine, 1:1, at room temperature) of **2** (16 mg) gave, after work-up and cc (Si gel), **5** (4 mg) as a solid: uv (MeOH) λ max (log ε) 263 (3.60) nm; ir (KBr) ν max 3449, 2996, 2936, 2876, 1736, 1709, 1678, 1649, 1459, 1377, 1244, 1172, 1096, 1021, 966, 901 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 4.94 (1H, br s, H-3), 2.88 (1H, d, J=13.4 Hz, H-12β), 0.66 (3H, s, H_3-18), 1.30 (3H, s, H_3-19), 0.90 (3H, s, H_3-21), 3.17 (1H, m, H-25), 1.27 (3H, d, J=7.0 Hz, H_3-27), 4.94 (2H, s, H_2-28), 0.85 (3H, d, J=6.7 Hz, H_3-29), 2.01 (3H, s, COMe); eims m/z [M]$^+$ 512 (100), 493 (9), 482 (17), 470 (52), 452 (90), 437 (31), 424 (25), 408 (17), 398 (43), 383 (26), 371 (32), 355 (41), 339 (19), 330 (40), 323 (13), 305 (17), 297 (48), 279 (30), 264 (77), 257 (30), 243 (31), 227 (13), 215 (16), 203 (18), 189 (32), 175 (33).

Oxidation of zhankuic acid B [**2**].—To a solution of CrO_3 (20 mg) in a mixture of CH_2Cl_2 (0.25 ml) and pyridine (0.033 ml) was added **2** (7 mg), and the mixture was vigorously stirred for 30 min at room temperature. The reaction mixture was filtered with Celite and the filtrate was reduced and chromatographed (Si gel) to yield a residue, identical with **1** (tlc, ^{1}H-nmr, and ms data).

Zhankuic acid C [**3**].—Pale yellow needles, mp 164–168°; $[\alpha]^{25}$D +118° (c=0.125, $CHCl_3$); uv (MeOH) λ max (log ε) 270 (3.70) nm; ir (KBr) ν max 3435, 2973, 2937, 1711, 1674, 1459, 1380, 1218, 1236, 1060, 990, 901 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Tables 1 and 2; hreims m/z 486.2982 ($C_{29}H_{42}O_6$, calcd 486.2981); eims m/z [M]$^+$ 486 (100), 468 (5), 442 (26), 372 (3), 357 (4), 341 (7), 331 (9), 329 (10), 313 (13), 303 (18), 291 (9), 275 (16), 261 (15), 249 (11), 229 (5), 215 (5), 201 (6), 189 (8), 175 (11), 161 (8), 149 (12).

Zhankuic acid C diacetate [**6**].—Acetylation (Ac_2O-pyridine, 1:1, at room temperature) of **3** (70 mg) gave, after work-up and cc (Si gel), **6** (55 mg) as a solid: mp 115–122°; $[\alpha]^{25}$D +91° (c=0.16, $CHCl_3$); uv (MeOH) λ max (log ε) 266 (3.80) nm; ir (KBr) ν max 3451, 2971, 2937, 1737, 1682, 1648, 1460, 1439, 1375, 1242, 1226, 1183, 1092, 1029, 966, 910, 826 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.16 (1H, m, H-1α), 2.33 (1H, m, H-1β), 1.79 (2H, m, H_2-2), 4.96 (1H, br s, H-3), 1.80 (1H, m, H-4), 2.08 (1H, td, J=15.5 and 3.0 Hz, H-5), 2.46 (1H, dd, J=15.5 and 3.0 Hz, H-6α), 2.24 (1H, t, J=15.5 Hz, H-6β), 5.07 (1H, s, H-12β), 2.96 (1H, dd, J=12.2 and 7.3 Hz, H-14), 1.42 (1H, m, H-15), 2.58 (1H, m, H-15), 1.32 (1H, m, H-16), 1.98 (1H, m, H-16), 1.73 (1H, m, H-17), 0.70 (3H, s, H_3-18), 1.34 (3H, s, H_3-19), 1.42 (1H, m, H-20), 0.90 (3H, d, J=6.5 Hz, H_3-21), 1.15 (1H, , H-22), 1.55 (1H, m, H-22), 1.87 (1H, m, H-23), 2.15 (1H, m, H-23), 3.13 (1H, q, J=7.0 Hz, H-25), 1.28 (3H, d, J=7.0 Hz, H_3-27), 4.91 (1H, br s, H-28a), 4.96 (1H, br s, H-28b), 0.84 (3H, d, J=6.6 Hz, H_3-29), 2.01 (3H, s, COMe), 2.08 (3H, s, COMe); ^{13}C nmr ($CDCl_3$) δ 28.5 (t, C-1), 26.1 (t, C-2), 72.7 (d, C-3), 33.3 (d, C-4), 42.1 (d, C-5), 38.1 (t, C-6), 201.4 (s, C-7), 143.6 (s, C-8), 152.4 (s, C-9), 38.1 (s, C-10), 197.4 (s, C-11), 81.4 (t, C-12), 48.4 (s, C-13), 42.8 (d, C-14), 23.8 (t, C-15), 27.0 (t, C-16), 46.7 (d, C-17), 11.4 (q, C-18), 16.3 (q, C-19), 35.3 (d, C-20), 18.1 (q, C-21), 33.8 (t, C-22), 31.2, 31.6 (t, C-23), 147.9, 148.0 (s, C-24), 45.2, 45.4 (d, C-25), 179.6 (s, C-26), 16.2 (q, C-27), 111.5 (t, C-28), 15.1 (q, C-29), 170.5 (s, OCOMe), 169.5 (s, OCOMe), 21.1 (q, $OCOCH_3$), 20.7 (q, $OCOCH_3$); eims m/z [M]$^+$ 570 (100), 528 (26), 510 (24), 465 (12), 450 (15), 449 (18), 435 (12), 355 (37), 295 (65), 281 (25), 267 (28), 235 (31), 213 (22), 188 (18), 175 (23), 161 (22), 149 (24), 137 (27), 121 (24), 109 (48).

BIOLOGICAL TESTING.—The cytotoxicity of compounds against the P-388 tumor cell line was determined as described previously using mithramycin (IC_{50} 0.06 μg/ml) as a standard (12). Anticholinergic and antiserotonergic activities were determined according to Levy's method (13). The isolated guinea pig ileum was perfused in a organ chamber using Tyrode's solution (30 ml). As the peristalsis of the ileum

became normal, acetylcholine (3 μg) or serotonin (30 μg) was added to cause contraction. Relaxation amplitude (cm) was measured by administration of test samples with acetylcholine or serotonin. It was considered positive if relaxation exceeded 80%. Atropine (0.1 μg/ml) was used as a reference for the anticholinergic study and promethazine (2 μg/ml) was used as a standard in the antiserotonergic test.

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