Antitumor Agents, 138. Rotenoids and Isoflavones as Cytotoxic Constituents from Amorpha fruticosa

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J. Nat. Prod., **1993**, 56 (5), 690-698• DOI:
10.1021/np50095a005 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 5, pp. 690-698, May 1993

ANTITUMOR AGENTS, 138.[1] ROTENOIDS AND ISOFLAVONES AS CYTOTOXIC CONSTITUENTS FROM *AMORPHA FRUTICOSA*

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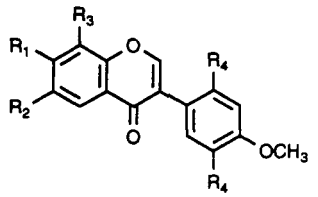
ABSTRACT.—Eight cytotoxic compounds have been isolated from the $CHCl_3$ extract of *Amorpha fruticosa*. One compound, 6′-*O*-D-β-glucopyranosyldalpanol [**10**], is a new cytotoxic rotenoid. Another known rotenoid, 12aβ-hydroxyamorphigenin [**6**], was first shown to exhibit extremely potent cytotoxicity ($ED_{50} < 0.001$ μg/ml) in six neoplastic cell lines. In addition to these compounds, three isoflavones (afrormosin [**1**], 7,2′,4′,5′-tetramethoxyisoflavone [**2**], 8-methylretusin [**3**]) and five rotenoids (amorphispironone [**4**], amorphigenin [**5**], dalpanol [**7**], 12aβ-hydroxydalpanol [**8**], and tephrosin [**9**]) were isolated. Compound **8** was isolated for the first time as a natural product from this plant. The structures of these compounds were established on the basis of spectral data; some were further confirmed by X-ray crystallographic analysis.

In the course of our continuing search for novel cytotoxic antitumor compounds from natural sources, the $CHCl_3$ extract of the aerial parts of *Amorpha fruticosa* L. (Leguminosae) was found to show cytotoxicity ($ED_{50} < 10$ μg/ml) against various tumor cell lines. Further bioassay-directed fractionation led to the isolation and characterization of three isoflavones and seven rotenoids. We have reported previously (1,2) the structure, stereochemistry, and chemical conversion of compound **4**, amorphispironone, a novel cytotoxic spironone-type rotenoid. In this paper, we report the isolation and characterization of the remaining compounds.

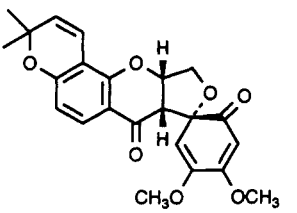
Compounds **1**, **2**, **3**, **5**, **6**, **7**, and **9** were identified as afrormosin (3), 7,2′,4′,5′-tetramethoxyisoflavone (4), 8-methylretusin (5), amorphigenin (6), 12aβ-hydroxyamorphigenin (7), dalpanol (8), and tephrosin (6), respectively, based on comparison with reported mp, uv, ir, ^{1}H- and ^{13}C-nmr, and ms data. The structures of **1**, **2**, and **5** were further confirmed by single-crystal X-ray analysis. From this plant, the two known natural products **1** and **3** were isolated for the first time.

The ^{1}H- and ^{13}C-nmr spectral data for compounds **5**, **7**, **8**, and **10** are given in Ta-

[1]For part 137, see K.F. Bastow, I.D. Bori, Y. Fukushima, Y. Kashiwada, G. Nonaka, I. Nishioka, and K.H. Lee, *Planta Med.*, in press.



1 R_1=OH, R_2=OMe, R_3=R_4=H
2 R_1=OMe, R_2=R_3=H, R_4=OMe
3 R_1=OH, R_2=R_4=H, R_3=OMe



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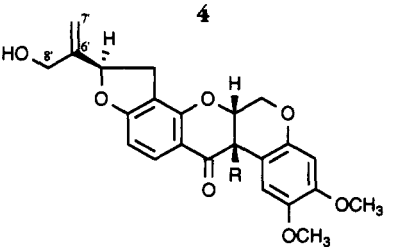
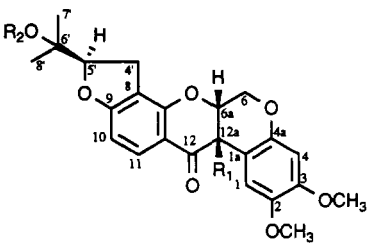


5 R=H
6 R=OH



7 R_1=R_2=H
8 R_1=OH, R_2=H
10 R_1=H, R_2=β-D-glucopyranosyl

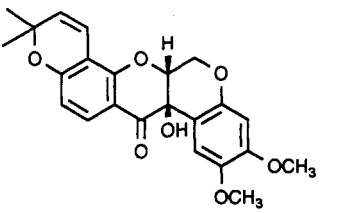


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TABLE 1. ^{1}H nmr Data of Rotenoids **5**, **7**, **8**, and **10** (ppm in $CDCl_3$, *J* in Hertz).

Proton	Compound			
	5	**7**	**8**	**10**
H-1	6.76 (s)	6.76 (s)	6.55 (s)	6.75 (s)
H-4	6.46 (s)	6.45 (s)	6.49 (s)	6.44 (s)
H-6	4.18 (d, *J* = 12)	4.18 (d, *J* = 12)	4.49 (d, *J* = 13)	4.18 (d, *J* = 12)
	4.61 (dd, *J* = 3, 12)	4.62 (dd, *J* = 3, 12)	4.62 (dd, *J* = 2.5, 13)	4.62 (dd, *J* = 3, 12)
H-6a	4.93 (m)	4.93 (m)	4.59 (t, *J* = 2.5)	4.94 (m)
H-10	6.51 (d, *J* = 8.5)	6.48 (d, *J* = 8.5)	6.52 (d, *J* = 8.5)	6.48 (d, *J* = 8.5)
H-11	7.85 (d, *J* = 8.5)	7.83 (d, *J* = 8.5)	7.82 (d, *J* = 8.5)	7.82 (d, *J* = 8.5)
H-12a	3.85 (d, *J* = 4)	3.84 (d, *J* = 4)	—	3.85 (d, *J* = 4)
H-4′	3.07 (dd, *J* = 9, 15)	3.11 (d, *J* = 9)	3.09 (d, *J* = 9)	3.13 (d, *J* = 9)
	3.39 (dd, *J* = 9, 15)	3.12 (d, *J* = 9)	3.10 (d, *J* = 9)	3.16 (d, *J* = 9)
H-5′	5.39 (t, *J* = 9)	4.71 (t, *J* = 9)	4.68 (t, *J* = 9)	4.79 (t, *J* = 9)
H-7′	5.26 (s), 5.28 (s)	1.22 (s)[a]	1.22 (s)[a]	1.33 (s)[a]
H-8′	4.27 (br s)	1.35 (s)[a]	1.35 (s)[a]	1.34 (s)[a]
OMe	3.77 (s)	3.76 (s)	3.73 (s)	3.76 (s)
OMe	3.81 (s)	3.80 (s)	3.82 (s)	3.80 (s)
H-1″ of Glucose				4.57 (d, *J* = 7.5)

[a]Assignments may be interchanged.

bles 1 and 2, respectively. Compound **8** (chemical formula $C_{23}H_{24}O_8$ from the hrms), was identified as the 12a-OH derivative of dalpanol [7], a known rotenoid, based on the similarities of their ^{1}H- and ^{13}C-nmr spectra. Replacement of the H-12a of **7** with an OH group in **8** is reflected in the absence of a signal for H-12a and in the low-field shift of H-6 (δ 4.18 to 4.49) as well as the high-field shifts of H-6a (δ 4.93 to 4.59) and H-1 (δ 6.76 to 6.55). The coupling pattern of H-6a is also simplified. The ^{13}C-nmr spectrum of **8** shows the expected low-field shift of C-12a (δ_C 44.7 to 67.6) and C-6a (δ_C 72.3 to 76.0). These data suggested that **8** is the 12a-OH derivative of dalpanol [7]. This was further confirmed by its ir absorptions (KBr) at 3530 and 1640 cm^{-1}, indicating the presence of hydrogen-bonded OH and carbonyl groups at positions 12a and 12, respectively. The relative stereochemistry of **8** was established as 6a*S*,12a*S* (12aβ-OH) by X-ray crystallographic analysis. The biosynthetic relationship of **8** to other rotenoids, such as dalpanol [7], as well as its co-occurrence with **10** (vide infra), indicates that this also represents its absolute stereochemistry. Fractional atomic coordinates for **8** are listed in Table 3, while a view of the solid-state conformation is presented in Figure 1. Bond lengths are in accord with expectations (9). Rings A and D are planar; ring B has a half-chair conformation with its C_2 symmetry axis passing through the mid-points of the C-1a–C-4a and C-6–C-6a bonds; ring C has a distorted half-boat (en-

TABLE 2. ^{13}C nmr Data of Rotenoids **5**, **7**, **8**, and **10** (ppm in $CDCl_3$)[a].

Carbon	Compound			
	5	**7**	**8**	**10**
C-1	110.5 (d)	110.5 (d)	*109.4* (d)	110.5 (d)
C-2	144.0 (s)	144.0 (s)	144.0 (s)	143.9 (s)
C-3	149.6 (s)	149.6 (s)	151.1 (s)	149.5 (s)
C-4	101.0 (d)	101.0 (d)	100.1 (d)	101.0 (d)
C-4a	147.5 (s)	147.5 (s)	148.4 (s)	147.4 (s)
C-6	66.3 (t)	66.3 (t)	63.9 (t)	66.2 (t)
C-6a	72.3 (d)	72.3 (d)	*76.0* (d)	72.2 (d)
C-7a	158.0 (s)	157.7 (s)	157.7 (s)	157.8 (s)
C-8	113.5 (s)	113.5 (s)	113.9 (s)	113.4 (s)
C-9	166.9 (s)	167.2 (s)	167.8 (s)	167.0 (s)
C-10	104.8 (d)	104.8 (d)	104.9 (d)	104.8 (d)
C-11	130.0 (d)	129.9 (d)	129.9 (d)	129.9 (d)
C-12	189.0 (s)	189.0 (s)	*191.2* (s)	189.0 (s)
C-12a	44.7 (d)	44.7 (d)	*67.6* (s)	44.5 (d)
C-1a	104.8 (s)	105.0 (s)	105.2 (s)	104.6 (s)
C-4′	31.9 (t)	27.4 (t)	27.3 (t)	27.8 (t)
C-5′	85.6 (d)	91.6 (d)	91.6 (d)	90.1 (d)
C-6′	146.8 (s)	71.7 (s)	71.8 (s)	*78.8* (s)
C-7′	112.9 (t)	26.2 (q)[b]	26.2 (q)[b]	*22.7* (q)[b]
C-8′	62.9 (t)	24.1 (q)[b]	24.1 (q)[b]	*21.5* (q)[b]
OMe	56.4 (q)	56.4 (q)	56.5 (q)	56.4 (q)
OMe	55.9 (q)	55.9 (q)	55.9 (q)	55.8 (q)
D-Glucosyl				
C-1″				97.2 (d)
C-2″				75.5 (d)
C-3″				77.2 (d)
C-4″				69.7 (d)
C-5″				76.4 (d)
C-6″				61.8 (t)

[a]The assignments were based on ^{13}C-^{1}H 2D nmr and by comparison with literature data (4).
[b]Assignments may be interchanged.

TABLE 3. Non-hydrogen Atom Fractional Coordinates and Equivalent Isotropic Thermal Parameters for **8**, with Estimated Standard Deviations in Parentheses.

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
C-1	0.5559(1)	0.2779(1)	−0.0708(2)	2.74(3)
C-1a	0.5089(1)	0.2694(1)	0.0807(3)	2.79(3)
C-2	0.6244(1)	0.3291(1)	−0.0783(3)	2.84(3)
C-3	0.6446(1)	0.3768(1)	0.0647(3)	3.18(3)
C-4	0.5992(1)	0.3680(1)	0.2148(3)	3.46(4)
C-4a	0.5337(1)	0.3128(1)	0.2227(2)	3.14(3)
O-5	0.4960(1)	0.3064(1)	0.3802(2)	4.06(3)
C-6	0.4430(1)	0.2376(2)	0.4037(3)	4.13(4)
C-6a	0.3846(1)	0.2277(1)	0.2538(3)	3.35(3)
O-7	0.3385(1)	0.3019(1)	0.2476(2)	3.43(3)
C-7a	0.2952(1)	0.3178(1)	0.1019(3)	2.78(3)
C-8	0.2352(1)	0.3778(1)	0.1120(3)	3.03(3)
C-9	0.1856(1)	0.3932(1)	−0.0299(3)	3.41(4)
C-10	0.1941(1)	0.3528(1)	−0.1844(3)	3.59(4)
C-11	0.2566(1)	0.2959(1)	−0.1936(3)	3.33(3)
C-11a	0.3085(1)	0.2769(1)	−0.0525(3)	2.85(3)
C-12	0.3767(1)	0.2189(1)	−0.0655(3)	3.03(3)
C-12a	0.4354(1)	0.2119(1)	0.0913(3)	3.07(3)
C-13	0.6580(1)	0.2939(1)	−0.3637(3)	3.84(4)
C-14	0.7335(2)	0.4766(2)	0.1856(4)	6.21(6)
O-2	0.6762(1)	0.3399(1)	−0.2167(2)	3.51(3)
O-3	0.7100(1)	0.4284(1)	0.0418(2)	4.74(3)
O-12	0.3888(1)	0.1788(1)	−0.1938(2)	4.11(3)
O-12a	0.4615(1)	0.1311(1)	0.1088(3)	4.16(3)
C-4′	0.2109(1)	0.4319(1)	0.2552(3)	3.81(4)
C-5′	0.1404(1)	0.4830(1)	0.1732(4)	3.97(4)
O-5′	0.1268(1)	0.4505(1)	−0.0008(2)	4.72(3)
C-6′	0.0570(1)	0.4818(1)	0.2688(4)	3.92(4)
O-6′	0.0351(1)	0.4000(1)	0.2910(3)	5.56(4)
C-7′	−0.0111(2)	0.5261(2)	0.1708(5)	5.58(6)
C-8′	0.0694(2)	0.5193(2)	0.4449(4)	5.41(5)

velope) form with C-6a as the out-of-plane atom; and ring E is quite flat. In crystals of **8**, both OH groups participate in intermolecular O-H . . . O hydrogen bonds [O-6′ . . . O-12 = 2.771(2) Å, O-12a . . . O-5′ = 3.069(2) Å] involving molecules related by the crystallographic 2_1 screw axis along the *a* direction. Compound **8**, 12aβ-hydroxydalpanol, has not been previously isolated as a natural product. However, it has been identified as an artificial metabolite from microbial transformations (8) of rotenone.

The ^{1}H-nmr spectrum of compound **10** (chemical formula $C_{29}H_{34}O_{12}$ $[MH]^+$ *m/z* 575), is also very similar to that of dalpanol [**7**] except for the presence of a more complicated pattern in the region 3.0 to 5.0 and a low-field shift of one of the isopropyl Me groups (C-7′, δ 1.22 to 1.33). In addition to carbon signals similar to those of **7**, the ^{13}C nmr of **10** shows six carbon signals belonging to glucose (Table 2). The ^{13}C nmr of **10** also shows a high-field shift of the two Me groups at C-7′ and C-8′ (δ_C 26.2 to 22.7 and 24.1 to 21.5) and a low-field of C-6′ (δ_C 71.8 to 78.8, glycosidation shift). These data suggested that the structure of **10** is 6′-*O*-glucopyranosyldalpanol. The anomeric proton signal of the sugar moiety in **10** was found at δ 4.57 (d, J = 7.5), indicating a β-D configuration. The complete structure of **10** was unambiguously established as 6′-*O*-β-D-glucopyranosyldalpanol by single-crystal X-ray analysis of the monohydrate. Car-

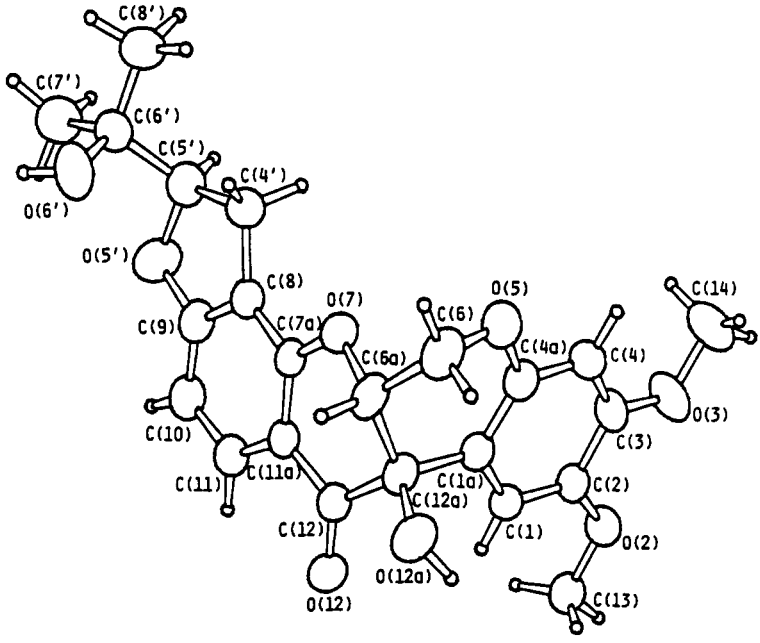

FIGURE 1. ORTEP diagram (50% probability ellipsoids) showing the atom numbering scheme and solid-state conformation of **8**; small circles represent hydrogen atoms.

bon and oxygen atom fractional coordinates are provided in Table 4. A view of the solid-state conformation is shown in Figure 2. Bond lengths are close to expected values (9) and agree well with corresponding distances in **8**. The conformations of rings A–E are similar to those in **8**, but there is less distortion of ring C from the corresponding half-boat (envelope) form with its C_s symmetry axis passing through C-6a and C-11a; ring E is slightly more puckered in **10** than in **8** and has a flattened envelope form with C-5′ as the out-of-plane atom. All OH groups and H_2O molecule hydrogen atoms are involved in intermolecular O-H . . . O hydrogen bonds [O(W) . . . O-2″ = 2.917 (3) Å, O-W . . . O-5′ (at $1-x$, $1/2+y$, $-z$) = 2.902 (4) Å, O-2″ . . . O-W (at $1-x$, $-\frac{1}{2}+y$, $-z$) = 2.911 (4) Å, O-3″ . . . O-6″ (at $1-x$, $\frac{1}{2}+y$, $-1-z$) = 2.807 (4) Å, O-4″ . . . O-5″ (at $1-x$, $\frac{1}{2}+y$, $-1-z$) = 2.941 (3) Å, O-6″ . . . O-W (at x, y, $-1+z$) = 2.803 (4) Å].

Table 5 shows the cytotoxicity data found for all ten compounds against six tumor cell lines. Two (**1** and **3**) of the three isoflavones were inactive in all assay systems, while 7,2′,4′,5′-tetramethoxyisoflavone [**2**] showed potent cytotoxicity against RPMI-7951, KB, and P388 cell lines. Isoflavone **2** has previously shown estrogenic activity (10). It is rare for an isoflavone to show strong cytotoxicity; however, biochain A, a 5,7-dihydroxy-4′-methoxyisoflavone, does show such an activity (11).

Compound **6**, 12aβ-hydroxyamorphigenin, demonstrated potent, but nonselective, cytotoxicity ($ED_{50}<0.001$ μg/ml) against all six neoplastic cell lines. Amorphigenin [**5**] was also very active in all assays but its activity was lower than its hydroxylated analogue **6**. Tephrosin [**9**], a structurally similar compound, also showed potent cytotoxicity in all assays. Dalpanol [**7**] and 12aβ-hydroxydalpanol [**8**], however, which contain an isopropanol side chain rather than the unsaturated alcohol found in **5** and **6**, were less potent than **5** and **6**, in general. The glucosyl derivative **10** of dalpanol was only weakly cytotoxic. Amorphispironone [**4**], a novel spironone-type rotenoid possess-

TABLE 4. Non-hydrogen Atom Fractional Coordinates and Equivalent Isotropic Thermal Parameters for **10**·H_2O, with Estimated Standard Deviations in Parentheses.

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
C-1	−0.0592(2)	−0.1342(5)	0.2433(3)	3.59(7)
C-1a	−0.0578(2)	−0.1810(4)	0.1080(3)	3.12(6)
C-2	−0.1225(2)	−0.0461(5)	0.2701(3)	3.91(7)
C-3	−0.1868(2)	0.0000(4)	0.1612(3)	3.54(7)
C-4	−0.1868(2)	−0.0454(4)	0.0280(3)	3.45(7)
C-4a	−0.1230(2)	−0.1356(4)	0.0027(3)	3.18(6)
O-5	−0.1294(1)	−0.1721(3)	−0.1348(2)	3.66(5)
C-6	−0.0807(2)	−0.2942(4)	−0.1595(3)	3.50(7)
C-6a	0.0089(2)	−0.2872(4)	−0.0762(3)	3.01(6)
O-7	0.0456(1)	−0.1593(3)	−0.1245(2)	2.99(4)
C-7a	0.1233(2)	−0.1212(4)	−0.0503(3)	2.51(5)
C-8	0.1739(2)	−0.0368(4)	−0.1164(3)	2.61(5)
C-9	0.2527(2)	0.0034(4)	−0.0447(3)	2.98(6)
C-10	0.2854(2)	−0.0307(4)	0.0945(3)	3.34(6)
C-11	0.2341(2)	−0.1149(4)	0.1582(3)	3.16(6)
C-11a	0.1536(2)	−0.1607(4)	0.0894(3)	2.84(6)
C-12	0.0989(2)	−0.2418(4)	0.1619(3)	3.45(7)
C-12a	0.0112(2)	−0.2830(4)	0.0798(3)	3.16(6)
C-13	−0.0625(4)	−0.0294(9)	0.5114(5)	8.5(2)
C-14	−0.3141(2)	0.1321(5)	0.0834(4)	4.09(7)
O-2	−0.1289(2)	0.0000(−)[a]	0.4000(3)	6.15(8)
O-3	−0.2472(1)	0.0889(4)	0.1949(2)	4.47(6)
O-12	0.1196(2)	−0.2730(4)	0.2838(3)	5.80(7)
C-4′	0.1604(2)	0.0148(4)	−0.2628(3)	3.14(6)
C-5′	0.2416(2)	0.1051(4)	−0.2593(3)	2.82(6)
O-5′	0.2975(1)	0.0780(3)	−0.1240(2)	3.66(5)
C-6′	0.2898(2)	0.0717(4)	−0.3747(3)	2.74(5)
C-7′	0.3173(2)	−0.0843(4)	−0.3744(4)	3.99(7)
C-8′	0.2335(2)	0.1142(5)	−0.5142(3)	4.07(8)
C-1″	0.3697(2)	0.2983(3)	−0.3757(3)	2.57(5)
C-2″	0.4447(2)	0.3697(4)	−0.2792(3)	2.56(5)
C-3″	0.4493(2)	0.5262(4)	−0.3222(3)	2.62(5)
C-4″	0.4538(2)	0.5369(3)	−0.4745(3)	2.43(5)
C-5″	0.3792(2)	0.4566(4)	−0.5621(3)	2.71(5)
C-6″	0.3797(2)	0.4509(4)	−0.7154(3)	3.52(7)
O-1″	0.3695(1)	0.1519(2)	−0.3430(2)	2.59(4)
O-2″	0.4348(2)	0.3659(3)	−0.1390(2)	3.99(5)
O-3″	0.5222(2)	0.5893(3)	−0.2343(2)	3.99(5)
O-4″	0.4482(1)	0.6838(3)	−0.5181(2)	3.56(5)
O-5″	0.3812(1)	0.3091(2)	−0.5153(2)	2.69(4)
O-6″	0.4554(2)	0.3882(3)	−0.7398(2)	4.23(5)
O-W	0.5189(2)	0.5655(3)	0.0754(2)	4.01(5)

[a]The *y*-coordinate of O-2 was held constant throughout the least-squares refinement to define the space group origin in this direction.

ing an unusual spiro A-B ring system, showed potent (ED_{50} < 1.0 μg/ml) and selective cytotoxicity against RPMI-7951 and KB tumor cell lines. The insecticidal activity of rotenoids has been regarded as their most important biological activity (10). Therefore, it was very interesting to find that these rotenoids demonstrated potent cytotoxicity against various tumor cell lines. Another example is rotenone, which was reported previously to be a cytotoxic agent against the growth of tissue culture cells derived from human epidermoid carcinoma of the larynx (H. Ep.-2) (12). Rotenone also demonstrated nonspecific cytotoxicity against various tumor cell lines (13).

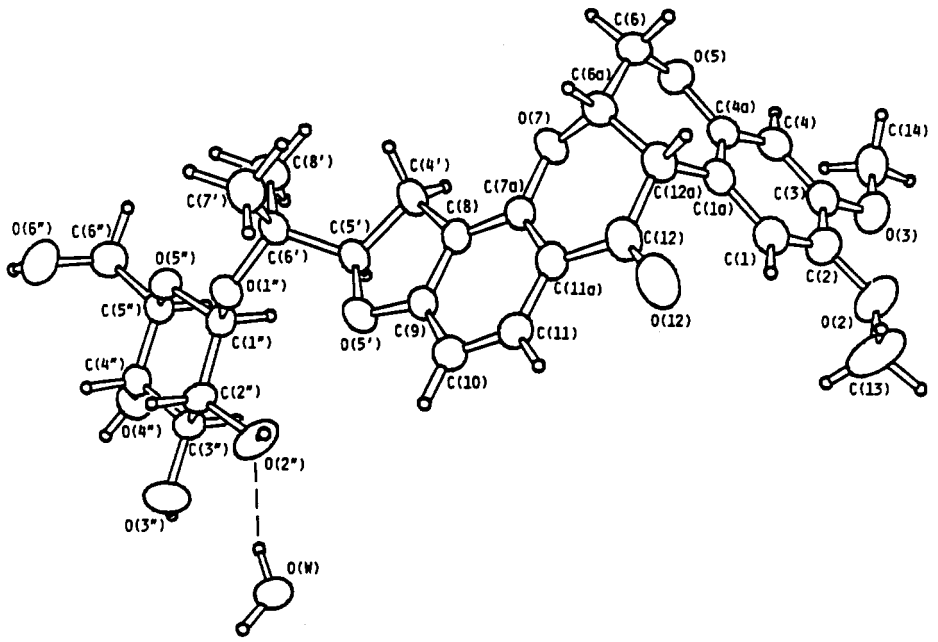

FIGURE 2. ORTEP diagram (50% probability ellipsoids) showing the atom numbering scheme and solid-state conformation of **10** in crystals of the monohydrate; small circles represent hydrogen atoms.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were taken on a Fischer-Johns mp apparatus and are uncorrected. Optical rotations were measured on an Autopol III automatic polarimeter. Ir spectra were recorded on a Perkin-Elmer 1320 spectrometer. ^{1}H- and ^{13}C-nmr spectra were obtained on a Bruker AC-300 spectrometer. All chemical shifts are reported in ppm from TMS. Ms was determined on a VG70-250SEQ mass spectrometer. Si gel (Aldrich Si gel 60, 5–25 μ) was used for medium-pressure cc, and reverse Si gel (Bondapak C_{18}, Merck) was employed for reverse cc. Analytical tlc was carried out on precoated Si gel (Merck 60 F-254) plates. Detection of components was performed by spraying with a solution of *p*-anisaldehyde–MeOH–H_2SO_4 (5:90:5). The cytotoxicity assay was carried out according to literature methods (14–16). Elemental analysis was performed on a Perkin-Elmer 2400 instrument.

TABLE 5. Cytotoxicity (ED_{50}, μg/ml) of Compounds **1–10** Against Human Cancer Cell Lines[a].

Compound	Cell Line					
	A-549	HCT-8	RPMI-7951	TE671	KB	P388
1	—[b]	—	—	—	—	—
2	—	4.63	0.49	—	0.55	0.53
3	—	—	—	—	—	—
4	—	—	0.61	—	0.58	—
5	0.05	0.03	0.05	<0.01	0.04	0.04
6	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
7	0.86	0.48	4.83	0.49	0.89	—
8	9.28	0.56	0.73	0.12	3.67	0.59
9	<0.001	0.09	0.07	0.05	0.36	0.06
10	—	7.43	4.44	2.95	6.32	6.68

[a]The bioassays were conducted according to literature methods (14–16).
[b]— = Inactive at 10 μg/ml).

PLANT MATERIAL.—The plants of *A. fruticosa* used in this investigation were collected at Fort Sill Military Reservation, Oklahoma, in August 1990. A voucher specimen was collected by Dr. J.R. Estes and is kept at the Department of Botany, The University of Oklahoma at Norman.

EXTRACTION AND FRACTIONATION.—The air-dried and ground aerial plant material (3.4 kg) was refluxed with MeOH (4 times, 4 h for each extraction) and evaporated in vacuo to give a residue. This residue was suspended in H_2O and extracted with *n*-hexane and $CHCl_3$. The $CHCl_3$ solution was concentrated to give a $CHCl_3$ fraction (57 g).

ISOLATION.—The isolation process was guided by the results of in vitro cytotoxicity bioassays. The active $CHCl_3$ extract was chromatographed with $CHCl_3$ and increasing proportions of MeOH to give six fractions. The first fraction was rechromatographed over Si gel with a gradient of hexane-Et_2O (1:1, 1:2, 1:3) to afford three pure compounds **1** (14 mg), **2** (26 mg), and **3** (10 mg), and impure **4** which was further purified using reverse cc with MeOH-H_2O (1:1) as an eluent to give pure **4** (750 mg). The second fraction was subjected to repeated cc over Si gel using $CHCl_3$/MeOH (gradient 1 to 4%) as eluent to yield **5** (17 mg), **6** (10 mg), **7** (28 mg), **8** (26 mg), and **9** (20 mg). The most polar fraction from the initial column was chromatographed over Si gel employing $CHCl_3$-MeOH (50:1) as eluent to give **10** (32 mg). For the identification of compounds **1–7** and **9** was previously described in the text.

12a-Hydroxydalpanol [**8**].—White crystalline needles: hrms $[M]^+$ 428.1481 (calcd 428.1474 for $C_{23}H_{24}O_6$); mp 206.5–207°; $[\alpha]^{20}D$ −250.9° (c = 0.055, MeOH); uv λ max (MeOH) (log ε) 289.4 (5.27), 267.8 (5.41), 248.8 (5.26); ir (KBr) 3430 (OH), 1640 (C=O), 1508, 1450, 1210, 1080 cm^{-1}; eims *m/z* $[M]^+$ 428; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

6′-*O*-β-D-*Glucopyranosyldalpanol* [**10**].—White crystalline needles: mp 162–162.5°; $[\alpha]^{20}D$ −106.7° (c = 0.045, dioxane). *Anal.* calcd for $C_{29}H_{34}O_{12}$. C 60.06, H 5.96; found C 59.83, H 6.03. Uv λ max (MeOH) (log ε) 291 (5.26); ir (KBr) 3420 (OH), 1660 (C=O), 1600, 1508, 1450, 1090 cm^{-1}; fabms *m/z* $[MNa]^+$ 597, $[MH]^+$ 575; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

X-RAY CRYSTAL STRUCTURE ANALYSIS OF 12aβ-HYDRIXTDAKOABIK [**8**] AND 6′-*O*-β-D-GLUCOPYRANOSYLDALPANOL [**10**]·H_2O[2].—*Crystal data for* **8**.—$C_{23}H_{24}O_8$, MW = 428.44, orthorhombic, space group $P2_12_12_1$, a = 15.813 (1), b = 16.778 (1), c = 7.818 (1) Å (from 25 orientation reflections, 36°<θ<40°), V = 2074.2 Å^3, Z = 4, D_c = 1.372 g·cm^{-3}, μ(CuKα radiation, λ = 1.5418 Å) 8.3 cm^{-1}; crystal dimensions 0.18 × 0.18 × 50 mm.

Crystal data for **10**·H_2O.—$C_{28}H_{34}O_{12}$·H_2O, MW = 592.60, monoclinic, space group $P2_1$, a = 16.137 (2), b = 9.249 (1), c = 9.869 (1) Å, β = 102.22 (1)° (from 25 orientation reflections, 35°<θ<40°), V = 1439.6 (5) Å^3, Z = 2, D_c = 1.367 g·cm^{-3}, μ(CuKα radiation) = 8.7 cm^{-1}; crystal dimensions 0.06 × 0.16 × 0.20 mm.

Preliminary unit-cell parameters and space group information were derived in each case from oscillation and Weissenberg photographs. Intensity data were recorded on an Enraf-Nonius CAD-4 diffractometer [CuKα radiation, graphite monochromator; θ max = 75°, ω–2θ scans; scan width (0.70 + 0.14 tan θ)° for **8**, (0.80 + 0.14 tan θ)° for **10**·H_2O]. The data were corrected for the usual Lorentz and polarization effects; an empirical absorption correction (T_{max}:T_{min} = 1.00:0.92) was also applied to the data for **10**·H_2O. From totals of 2433 and 3147 non-equivalent measurements for **8** and **10**·H_2O, respectively, those 2189 and 2278 reflections with $I>3.0\sigma(I)$ were retained for the structure analyses.

Both crystal structures were solved by direct methods (MULTAN11/82). Approximate carbon and oxygen atom coordinates for **8** were obtained from an E-map whereas for **10**·H_2O they were derived in part from an *E*-map and from difference Fourier syntheses phased successively by an increasing number of atoms. Hydrogen atoms were located in difference Fourier syntheses evaluated following several rounds of full-matrix least-squares adjustment of non-hydrogen atom positional and thermal parameters (at first isotropic, then anisotropic). With the inclusion of hydrogen atom positional and isotropic thermal parameters as well as an extinction correction (g) as variables in the subsequent least-squares iterations, the parameter refinement converged at $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.033\{R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0.049$, $g = 1.6\ (1) \times 10^{-6}$, $GOF = [\Sigma w(|F_o| - |F_c|)^2/(N_{observations} - N_{parameters})]^{1/2} = 1.44\}$ for **8** and R = 0.034 [R_w = 0.045, $g = 1.4\ (2) \times 10^{-6}$, GOF = 1.08] for **10**·H_2O.

Crystallographic calculations were performed on PDP11/44 and Micro VAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from the literature (17). In the least-squares iterations, $\Sigma w\Delta^2[w = 1/\sigma^2(|F_o|), \Delta = (|F_o| - |F_c|)]$ was minimized.

[2]Atomic coordinates for **8** and **10**·H_2O have been deposited at the Cambridge Crystallographic Data Centre, and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the National Cancer Institute awarded to K.H. Lee (CA 17625).

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Received 13 August 1992