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GUMMIFEROL, A CYTOTOXIC POLYACETYLENE FROM THE LEAVES OF *ADENIA GUMMIFERA*

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ABSTRACT.—A new polyacetylenic diepoxide compound, gummiferol [**1**] was isolated from the leaves of *Adenia gummifera* by KB cytotoxicity-guided fractionation. Compound **1** exhibited significant activity against the KB human cell line and a broad cytotoxic spectrum against other human cancer cell lines. The structure of **1** was established primarily on the basis of its spectral data.

Adenia gummifera (Harv.) Harms (Passifloraceae) is a species that grows in various regions of Africa. The juice from the leaves of this plant is used in Tanzania to treat colic, while the crushed leaves are applied externally in the treatment of broken bones (1). The roots are employed as a diuretic, to treat filariasis, and against infertility (1–3). Various other traditional medicinal uses of the different parts of *A. gummifera* have also been reported (1). However, hepatic toxicity has been associated with the chronic traditional use of *A. gummifera* among the Zulu of South Africa (4). A literature survey revealed that this plant has not received any previous phytochemical investigation. The organic portion of a 20% MeOH/$CHCl_3$/H_2O partition of a 50% MeOH/$CHCl_3$ extract of the leaves of *A. gummifera* exhibited an ED_{50} value of 1.1 μg/ml in the human epidermoid carcinoma (KB) cell line assay. Si gel chromatographic fractionation and purification of the 20% MeOH/$CHCl_3$ extract afforded a new active polyacetylenic compound, gummiferol [**1**]. In this communication, we wish to report the isolation and structure elucidation of compound **1**.

The high-resolution fabms of gummiferol [**1**] indicated an elemental composition of $C_{16}H_{14}O_5$ (found *m/z* $[M+H]^+$ 287.0926, calcd 287.0919). The ir spectrum exhibited absorptions due to hydroxyl (3395 cm^{-1}) and ester (1695 cm^{-1}) functionalities. Furthermore, the absorptions at 1653 and 956 cm^{-1} were suggestive of a trans-double bond. The uv spectrum showed absorption bands for a conjugated triyne chromophore (λ max at 253, 269, 287, 307 nm). In the ^{13}C- and DEPT nmr spectra of **1** (Table 1), the presence of three triple bonds was corroborated by the resonances at δ 62.43 (C-4), 62.80 (C-5), 69.00 (C-6), 70.12 (C-3), 73.90 (C-7), and 77.20 (C-2). The ^{13}C-nmr sp^3 signals at δ 43.05, 51.31, 55.15, 56.20, 57.46, and 63.55 were due to C-8, C-1, C-11, C-10, C-9, and C-14, respectively. The remaining ^{13}C-nmr resonances at δ 129.37 and 130.53 were in turn attributable to the double bond sp^2 carbons (C-12 and C-13), while ester carbon signals occurred at δ 20.87 (CH_3) and 170.77 (C=O). In the ^1H-nmr spectrum of **1**, a primary alcohol methylene resonance appeared at δ 4.34 (H_2-1), while an allylic methylene occurred at δ 4.58 (H_2-14). The shielding of the ^1H-nmr

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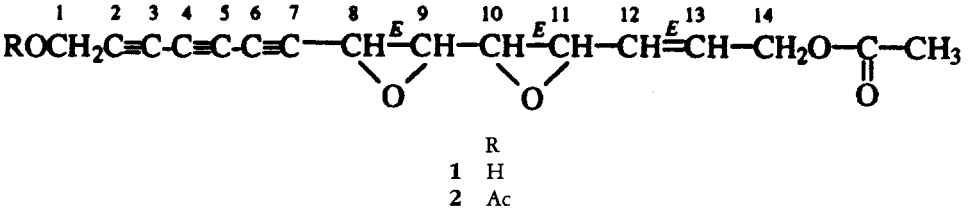

R
1 H
2 Ac

TABLE 1. ^{13}C-nmr Chemical Shifts of Gummiferol [**1**] and Gummiferol Acetate [**2**] (ppm, in $CDCl_3$).

Carbon	Compound	
	1	**2**
C-1	51.31	52.15
C-2	77.20	74.11
C-3	70.12	71.00
C-4	62.43	62.52
C-5	62.80	62.75
C-6	69.00	68.90
C-7	73.90	72.66
C-8	43.05	43.00
C-9	57.46	57.46
C-10	56.20	56.15
C-11	55.15	55.12
C-12	129.37	129.47
C-13	130.53	130.55
C-14	63.55	63.48
*C*OCH$_3$-14	170.77	170.57
COC*H*$_3$-14	20.87	20.86
*C*OCH$_3$-1	—	169.91
COC*H*$_3$-1	—	20.58

chemical shift of H_2-1 (Δ 0.41 ppm) and the ^{13}C-nmr chemical shift of C-1 (Δ 0.84 ppm), relative to gummiferol acetate [**2**], proved the presence of a primary alcohol group at C-1 in compound **1**. The oxirane protons of **1** resonated at δ 3.04 (H-10), 3.35 (H-9), 3.39 (H-11), and 3.46 (H-8), with the ^{1}H-nmr signals at δ 5.49 and 6.05 being readily assigned to H_2-1 and H-13, respectively. An ester methyl signal was evident at δ 2.09. A ^{1}H-^{1}H COSY nmr spectrum of **1** established the connectivity of H-8 through H_2-14. Thus, H-8 (δ 3.46) correlated with H-9 (δ 3.35) which in turn correlated with both H-8 and H-10 (δ 3.04). The H-10 proton was coupled to H-11 (δ 3.39), which was connected to the vinyl proton H-12 (δ 5.49), which in turn showed vicinal and allylic correlation to H-13 (δ 6.05) and H_2-14 (δ 4.58), respectively. The ^{1}H- and ^{13}C-nmr chemical shift assignments of **1** were facilitated by comparison with the corresponding chemical shift values of the acetate derivative **2**, and by HMQC (5) and HMBC (6) nmr experiments. Thus, in the HMQC spectrum of **1**, each of the protonated carbons was correlated to the respective directly attached proton(s). Long-range ^{1}H-^{13}C nmr correlations, primarily two-bond and three-bond, were evident in the HMBC spectrum of **1**. The most important long-range HMBC nmr correlations of **1** are indicated in Figure 1. The trans (*E*)-configuration at the C-12/C-13 double bond was inferred from the ^{1}H-nmr coupling constant of H-12 and H-13 (J=15.6 Hz). The small coupling constants (J=2.0 Hz) of the vicinal methine signals of the epoxy rings, as compared to the larger coupling constant of 4.0 Hz reported for similar cis-oriented protons (7,8) indicated the relative trans (*E*)-configuration of the epoxy rings in gummiferol [**1**]. Thus, the structure of gummiferol [**1**] was assigned as 14-*O*-acetyl-8,9(*E*)-10,11(*E*)-diepoxy-12(*E*)-en-2,4,6-triyntetradecan-1-ol.

As shown in Table 2, gummiferol was found to demonstrate a general cytotoxic response with no discernible cell-type selectivity.

Although a diverse array of polyacetylenes has been isolated mainly from species in the Compositae and Umbelliferae and to a lesser extent from plants in other families (9), the polyacetylene diepoxide structural feature of **1** appears to be unique. The closest structural variant of **1** is a C_{14}-monoepoxy

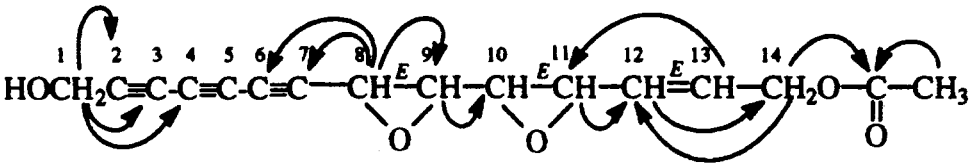

FIGURE 1. Key HMBC nmr correlations of **1**.

TABLE 2. Cytotoxicity of Gummiferol [**1**].

Cell Line[a]	ED_{50} μg/ml	Cell Line[a]	ED_{50} μg/ml
BC1	0.2	P-388	0.03
HT	0.1	A-431	0.5
Lu1	0.9	LNCaP	0.2
Mel2	1.3	ZR-75-1	0.2
Col2	0.6	U-373	0.05
KB	0.3	KB-V(−VLB)	0.4
KB-V(+VLB)	0.3		

[a]See Likhitwitayawuid *et al.* (10) for full details of cell lines.

acetylenic compound previously isolated from *Panax quinquefolium* (Araliaceae) as a cytotoxic constituent (8). The concomitant presence of high unsaturation and epoxide functionalities might well confer the observed cytotoxic activity on **1**. Despite the fact that polyacetylenes are in general known to be labile, **1** appeared to be fairly stable in our hands. However, if there had been prior knowledge of the occurrence of polyacetylenes, it would have been preferable to extract the plant material under milder conditions to avoid any possibility of isomerization and degradation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr experiments were performed on a Bruker AMX 500 spectrometer operating at 500 MHz for proton and 125 MHz for carbon using solutions in $CDCl_3$ with TMS as internal standard. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Uv spectra were recorded on a Varian 2290 uv-vis spectrometer, ir on a Perkin-Elmer 467 spectrometer, and hrfabms on an Associated Electrical Industries MS-902 instrument. Cc was carried out using Si gel 60 (70–230 mesh, E. Merck, Darmstadt, Germany). Tlc was performed on Merck aluminum-backed tlc sheets (Si gel F_{254}), with visualization using phosphomolybidate spray reagent (5% phosphomolybidic acid in EtOH). Prep. tlc was carried out on Merck Si gel F_{254} plates (1 mm thickness).

PLANT MATERIAL.—The leaves of *Adenia gummifera* were collected in Mazoe, Zimbabwe in February 1992. A voucher specimen representing this collection has been deposited at the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, Illinois.

EXTRACTION AND ISOLATION.—The ground leaves (450 g) were extracted with 50% MeOH/$CHCl_3$ under reflux. The concentrated extract was suspended in H_2O and partitioned with 20% MeOH/$CHCl_3$. After concentration, the organic phase was resuspended in 90% MeOH/H_2O and defatted with mutually saturated hexane. The aqueous MeOH portion (5.8 g) was chromatographed on a Si gel column and eluted with a $CHCl_3$/MeOH mixture of increasing polarity. Elution was monitored by tlc on Si gel F_{254} (Merck). The most active fraction (KB, ED_{50} 0.9 μg/ml; 1.06 g) was further chromatographed on a Si gel column, and subsequent prep. tlc (5% MeOH/$CHCl_3$) afforded pure gummiferol [**1**] (40 mg).

ACETYLATION OF GUMMIFEROL [**1**].—Gummiferol [**1**] (6 mg) was treated with Ac_2O (0.5 ml) and pyridine (1.0 ml) by stirring at room temperature for 3 h. After removal of the solvent, the residue was purified by prep. tlc (3% MeOH/$CHCl_3$) to give gummiferol acetate [**2**] (5 mg).

Gummiferol [**1**].—Off-white amorphous solid; $[\alpha]^{25}D$ −170° (c=0.2, MeOH); hrfabms m/z $[M+H]^+$ 287.0926 (requires 287.0919, Δ 2.3 ppm); uv (MeOH) λ max (log ε) 253 (3.10), 269 (3.24), 287 (3.28), 307 (3.09) nm; ir (KBr) ν max 3395, 3005, 1695, 1653, 1446, 1373, 1267, 1049, 1023, 956, 893, 850 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) δ 6.05 (1H, dt, J=15.6 and 5.7 Hz, H-13), 5.49 (1H, dd, J=15.6 and 7.8 Hz, H-12), 4.58 (2H, dd, J=5.7 and 1.3 Hz, H_2-14), 4.34 (2H, s, H_2-1), 3.46 (1H, d, J=2.0 Hz, H-8), 3.39 (1H, dd, J=7.8 and 2.0 Hz, H-11), 3.35 (1H, dd, J=3.1 and 2.0 Hz, H-9), 3.04 (1H, dd, J=3.1 and 2.0 Hz, H-10), 2.09 (3H, s, -COCH_3); ^{13}C-nmr data, see Table 1.

Gummiferol acetate [**2**].—Whitish-brown

solid; $[\alpha]^{25}D$ −153° (*c*=0.2, MeOH); hrfabms *m/z* $[M+H]^+$ 329.1023 (requires 329.1025, Δ −0.6 ppm); uv (MeOH) λ max (log ε) 254 (3.01), 269 (3.11), 287 (3.15), 307 (3.00), 318 (2.65) nm; ir (KBr) ν max 3005, 1727, 1670, 1432, 1377, 1240, 1029, 959, 910, 863, 689 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) δ 6.04 (1H, dt, *J*=15.6 and 5.7 Hz, H-13), 5.48 (1H, dd, *J*=15.6 and 7.8 Hz, H-12), 4.75 (2H, s, H_2-1), 4.58 (2H, dd, *J*=5.7 and 1.4 Hz, H_2-14), 3.45 (1H, d, *J*=2.0 Hz, H-8), 3.44 (1H, dd, *J*=3.1 and 2.0 Hz, H-9), 3.38 (1H, dd, *J*=7.8 and 2.0 Hz), 3.02 (1H, dd, *J*=3.1 and 2.0 Hz, H-10), 2.11 (3H, s, -COCH_3-1), 2.08 (3H, s, -COCH_3-14); ^{13}C-nmr data, see Table 1.

CYTOTOXICITY ASSAY.—Gummiferol [**1**] was tested for cytotoxicity in 13 mammalian cancer cell lines as described previously (10). The results are in Table 2. Chromatographic fractions were assayed for KB activity utilizing a 96-well microtiter plate as described previously (10).

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