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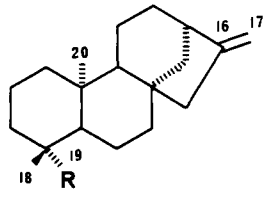
CHEMISTRY IN THE ANNONACEAE, XXIV.[1] KAURANE AND KAUR-16-ENE DITERPENES FROM THE STEM BARK OF *ANNONA RETICULATA*

JOSEPH T. ETSE,[2] ALEXANDER I. GRAY, and PETER G. WATERMAN*

Phytochemistry Research Laboratories, Department of Pharmacy (Pharm. Chem.), University of Strathclyde, Glasgow G1 1XW, Scotland, UK

Annona reticulata L. (Annonaceae), a tree of dry, tropical forests, probably originated in Central America and is now cultivated throughout the tropics (1). It has long been known as a source of 1-benzyltetrahydroisoquinoline alkaloids (2) and has recently been reported to yield an acetogenin (3). Several *Annona* species have yielded diterpenes (2), but they have not previously been found in *A. reticulata*. In this paper we report the isolation of several kaurane diterpenes, some of them novel, from the stem bark of this species.

Column chromatography of an EtOAc extract over Si gel eluting with petroleum ether containing increasing amounts of EtOAc yielded seven bands labeled A-G (Table 1). Identification of G as 14-hydroxy-25-desoxyrollinicin has been reported previously (3). Bands A and C were characterized as (−)-kaur-16-en-19-oic acid [**1**] and (−)-kauran-16α-ol [**2**] by direct comparison with material isolated from *Xylopia aethiopica* (4) and *Xylopia acutifolia* (5), respectively. Both have been reported previously from other *Annona* species (2). Band B was characterized as the sesquiterpene caryophyllene-4,5-oxide (6).



	R
1	COOH
8	$COOCH_3$

Band F recrystallized as levorotatory needles (yield 0.3%). The ir spectrum indicated the presence of ester carbonyl (1720 cm^{-1}) and hydroxyl group(s) (3400 cm^{-1}), the latter being confirmed by formation of a monoacetate and a diacetate. Eims revealed a molecular ion at *m/z* 392 ($C_{23}H_{36}O_5$) for the monoacetate. The highest *m/z* fragment found in the parent compound was 332 ($C_{21}H_{32}O_3$), requiring facile loss of the elements of H_2O from a molecular formula of $C_{21}H_{34}O_4$.

The ^{1}H-nmr spectrum revealed two tertiary methyl resonances at δ 1.16 and 0.83 and a methoxyl signal at δ 3.64. These are typical of the equatorial C-18 and axial C-20 methyl groups of a kaurane diterpene with a C-19 axial carboxylic acid methyl ester (7). This is supported by the frequency of the ir carbonyl band (7) and the ^{13}C-nmr resonances at 177.9 ppm (C-19), 28.6 (C-18), and 15.1 (C-20) (8). The other major feature of the ^{1}H-nmr spectrum was an AB quartet (2H) with doublets centered at δ 3.37 and 3.47 (J=11 Hz) suggesting the presence of a hydroxymethyl group. In the ^{1}H-nmr spectrum of the monoacetate these signals were deshielded to δ 3.90 and 4.07 and in the spectrum of the diacetate to a singlet at δ 4.40. This hydroxymethyl group must be derived from the C-17 methyl group and, in view of the absence of further coupling for H-17, the second hydroxyl group must be placed at C-16. The tertiary nature of the hydroxy group at C-

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[2]Present address: Chemistry Department, University of Georgia, Athens, Georgia 30602.

TABLE 1. Isolates Obtained by Si gel 60 Column Chromatography of the EtOAc Extract of *Annona reticulata*

Solvent[a]	Band	Yield (%)	Tlc[b]	Solvent[c]	Identity
100:0	—	50 mg	0.95	system 1	fats
98:2	A	900 mg	0.80	system 1	**1**
97:3	B	60 mg	0.75	system 1	caryophyllene-4,5-oxide
96:4	C	80 mg	0.60	system 1	**2**
92:8	D	150 mg	0.30	system 1	**12,13**
			0.70	system 2	
	D′	60 mg	0.60	system 2	**16,17**
90:10	E	350 mg	0.60	system 2	**16,17**
85:15	F	360 mg	0.50	system 2	**7**
0:100	G	55 mg	0.40	system 2	acetogenin (3)

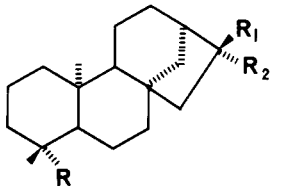
[a]Petroleum ether (bp 40-60°)-EtOAc.
[b]Si gel G.
[c]System 1=toluene-EtOAc-HOAc (8:2:1); system 2=toluene-EtOAc-HOAc (5:4:1).

16 was supported by its relative resistance to acetylation, the absence of an oxymethine signal in the ^{1}H nmr, and resonances at δ 79.8 (s) and 69.7 (t) in the ^{13}C nmr for C-16 and C-17, respectively.

On this evidence, band F must be a 16,17-dihydroxy derivative of methyl kauran-19-oate, and it remains only to resolve the stereochemistry at C-16. The 16α,17-dihydroxy methyl ester [**3**] has recently been reported (9) and is characterized by an AB-quartet with doublets centered at δ 3.66 and 3.78 (J=11 Hz) for H-17 which is different from that reported here for band F. Likewise in the spectrum of 16α,17-dihydroxykaurane [**4**] H-17 protons resonate at δ 3.65 and 3.80 (J=11 Hz). By contrast, in the spectrum of 16β,17-dihydroxykaurane [**5**] they occur at δ 3.37 and 3.51 (J=12 Hz) (10) and in that of 16β,17-dihydroxy-19-oic acid [**6**] at δ 3.32 and 3.43 (J=11 Hz), positions comparable to that observed here. On this basis the isolated compound can be identified as methyl 16β,17-dihydroxy-(−)-kauran-19-oate [**7**]. This compound appears to be novel but has recently been reported as a synthetic derivative of **6** (11).

In order to confirm the identity of **7**, attempts were made to synthesize it or its isomer **3** from the corresponding kaur-16-en-19-oate [**8**]. Treatment with chloroperoxybenzoic acid for 24 h did not give the anticipated 16α,17-epoxide (12) but a mixture of the 16α,17- and 16β,17-epoxides in a ratio of 3:2. Acid hydrolysis of this mixture gave the corresponding 17-aldehydes. By contrast oxidation of **1** with OsO_4 (13) gave only the 16α,17-diol, which on methylation gave **3**, with physical constants identical to literature data (9) but different from those of the isolated diterpene [**7**].

In the course of this study ^{13}C-nmr spectra were obtained for **7**, its acetates **9** and **10**, and the isomer **3**. Chemical



	R	R_1	R_2
2	Me	Me	OH
3	$COOCH_3$	CH_2OH	OH
4	Me	CH_2OH	OH
5	Me	OH	CH_2OH
6	COOH	OH	CH_2OH
7	$COOCH_3$	OH	CH_2OH
9	$COOCH_3$	OH	CH_2OAc
10	$COOCH_3$	OAc	CH_2OAc
11	COOH	CH_2OH	OH
12	$COOCH_3$	CH_2OH	H
13	$COOCH_3$	H	CH_2OH
14	CH_2OH	$COOCH_3$	H
15	CH_2OH	H	$COOCH_3$
16	COOH	CH_2OH	H
17	COOH	H	CH_2OH

shift values for these compounds, and previous data for **4**, **5**, and the 16α,17-hydroxy acid **11** are listed in Table 2. Chemical shifts for C-16 and C-17 readily distinguish the 16α- from the 16β-isomer.

and that at δ 3.40 with a resonance at δ 1.82-1.86, suggesting the presence of two $CH\text{-}CH_2OH$ systems. Band D must, therefore, consist of the 16-hydroxymethyl isomers **12** and **13**. From ^{1}H-nmr integrals it appears to be a 50:50

TABLE 2. ^{13}C-nmr Chemical Shift Values for Diterpenes[a]

Carbon No.	Compounds							
	4(10)	**11**(8)	**3**	**7**	**5**(10)	**9**	**10**	**12/13**
1	42.0	41.1	42.1	41.8	41.4	41.2	41.5	41.6/42.0
2	18.2	19.8	19.1	19.0	18.7	18.9	19.0	19.0
3	42.0	38.7	38.1	38.0	42.0	38.0	37.9	38.0
4	33.4	43.9	43.9	43.7	33.2	43.5	43.7	44.1/44.6
5	56.1	57.0	56.9	56.8	56.1	56.7	56.7	56.6/56.9
6	20.5	22.9	22.2	21.5	20.0	21.5	21.6	22.1/22.4
7	37.2	42.7	40.7	40.6	38.2	40.5	40.6	40.7/40.8
8	44.6	44.9	44.7	43.4	43.5	43.6	43.2	43.6/43.7
9	56.7	56.3	55.8	56.0	56.9	55.9	55.8	55.3/56.4
10	39.4	40.0	39.5	39.4	39.3	39.3	39.3	39.3/39.8
11	18.3	18.9	18.6	18.9	18.6	18.9	18.9	18.3/18.8
12	26.3	26.8	26.2	26.5	26.7	26.4	26.8	26.0/28.9
13	45.5	45.8	45.4	40.5	52.6	41.7	41.7	37.1
14	40.4	37.8	37.3	37.9	40.4	37.8	37.0	38.1/40.3
15	53.4	53.9	53.2	52.3	56.1	52.3	50.4	43.6/44.9
16	81.6	81.6	81.9	79.8	79.7	78.4	86.9	43.2/42.3
17	66.2	66.4	66.4	69.7	69.7	71.6	66.0	64.1/67.4
18	33.4	29.3	28.7	28.6	33.6	28.6	28.6	31.3
19	21.5	180.1	177.9	177.9	21.5	177.9	177.9	177.9
20	17.7	15.4	15.4	15.1	17.6	15.4	15.1	18.7
OMe			51.1	51.0		51.0	51.0	51.0
Ac						20.7	21.6/20.7	
						171.1	170.5/170.7	

[a]Spectra run at 62.5 MHz or 90.56 MHz.

Band D gave a levorotatory solid (yield 0.15%). Eims indicated a molecular formula, $C_{21}H_{34}O_3$, and ir and ^{1}H-nmr spectra revealed a similar 19-carboxymethyl system to that of **7**. The other major feature of the ^{1}H-nmr spectrum was the presence of doublets at δ 3.40 and 3.70 (both J=7 Hz). These were deshielded by 0.45 ppm on acetylation. A ^{13}C-nmr spectrum showed the presence of more than 21 signals which indicated a mixture of compounds. Detailed tlc examination failed to resolve the mixture, but decoupling experiments in the ^{1}H nmr revealed that there was no interaction between the two deshielded doublets, that at δ 3.70 interacting with a resonance at δ 2.10-2.16

mixture. The alternative structures **14** or **15** can be discounted as acetylation caused no change in ^{1}H-nmr chemical shifts for C-18 or C-20. Comparison with published data (7) allows the oxymethylene resonance at δ 3.40 to be assigned to the 16β-hydroxymethyl isomer **12**.

Diterpenes **12** and **13** are new natural products but have previously been reported through methylation of the corresponding acids **16** and **17**. These isomeric acids were the final diterpenes to be isolated (combined yield 0.4% from band E). Their identity was established by conversion to the corresponding methyl esters. The 16α-hydroxymethyl acid **17** has been found in the

Euphorbiaceae (14) and Compositae (15,16), and the 16β-hydroxymethyl acid **16** is known from the Compositae (17).

Both **1** and **2** have previously been reported from other *Annona* species (2), but the remaining compounds are all new, at least to the Annonaceae. The occurrence of **12/13** and **16/17** as 50:50 mixtures is unusual, particularly as **7**, which is presumably formed from the same 16,17-oxide intermediate, occurs as the single isomer. It may be that **7** is the product of an enzyme controlled biogenetic sequence whereas **12/13** and **16/17** are products of nonenzymatic fission of the 16,17-oxide precursor.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Ir spectra were measured as KCl discs. ^{1}H-nmr spectra were run at 250 MHz or 360 MHz and ^{13}C-nmr spectra at 62.5 MHz or 90.56 MHz, in $CDCl_3$ using TMS as internal standard. High resolution eims were obtained on an AEI MS902 double focusing instrument by direct probe insert at elevated temperatures and 70 eV.

PLANT MATERIAL.—Stem bark of *A. reticulata* was collected from the Santa Rosa National Park, Guanacaste, Costa Rica, and a voucher specimen has been preserved at the Herbarium of the Missouri Botanic Gardens, St. Louis, Missouri, as part of a general collection of plants from the Park.

ISOLATION OF COMPOUNDS.—Ground stem bark (400 g) was macerated with ammoniacal MeOH for 5 days. The extract was filtered, evaporated to dryness, and the resulting solid (100 g) refluxed with EtOAc. The EtOAc-soluble portion was subjected to column chromatography over Si gel 60 (Table 1). Five bands, A, B, D, D′+E, and F, were subsequently purified by preparative circular tlc on Si gel G with solvent mixtures as follows: A, toluene-EtOAc (96:4) to give **1**, 80 mg; B, toluene-EtOAc (95:5) to give caryophyllene-4,5-oxide, 40 mg; D, toluene-EtOAc-HOAc (92:8:1) to give **12**, **13**, 140 mg; D′+E, toluene-EtOAc-HOAc (90:10:1) to give **16**, **17**, 360 mg; F, toluene-EtOAc-HOAc (87:12:1) to give **7**, 300 mg.

(−)-*Kaur-16-en-19-oic acid* [**1**].—Cubes from $CHCl_3$, mp 171-173°, $[\alpha]^{25}D$ −110° (*c* 0.1, $CHCl_3$); eims (*m/z*) 302.2235 (calcd $C_{20}H_{30}O_2$, 302.2246). Treatment with CH_2N_2 yielded the methyl ester [**8**], mp 88-89°; eims (*m/z*) 316.2385 (calcd $C_{21}H_{32}O_2$ 316.2402). Both **1** and its methyl ester were identical with an authentic sample (ir, ^{1}H nmr, eims, mixed mp, or) (4).

(−)-*Kauran-16α-ol* [**2**].—Plates from petroleum ether/EtOAc, mp 214-215°, $[\alpha]^{25}D$ −40° (*c* 0.06, $CHCl_3$); eims (*m/z*) 290.2624 (calcd $C_{20}H_{34}O$ 290.2610). Identical (by ir, ^{1}H nmr, eims, mixed mp, or) with an authentic sample (5).

Methyl 16β,17-dihydroxy-(−)-kauran-19-oate [**7**].—Needles from $CHCl_3$/MeOH, mp 118-120°, $[\alpha]^{25}D$ −95° (*c* 0.06, $CHCl_3$); ir max 3400, 1720 cm^{-1}; ^{1}H nmr δ 0.83 (s, 3H, 20-CH_3), 1.16 (s, 3H, 18-CH_3), 3.37, 3.47 (ABq, 2H, J=11 Hz, 17-CH_2), 3.64 (s, 3H, OCH_3); ^{13}C nmr see Table 2; eims (*m/z*, rel. int.) 332.2359 (M^+-H_2O, 1%) (calcd for $C_{21}H_{32}O_3$ 332.2351), 319 (M^+-OCH_3, 100%), 289 (40%), 273 (5%), 259 (27%), 123 (18%), 121 (16%), 109 (18%), 107 (18%).

Acetates of **7**.—Compound **7** (60 mg) was dissolved in pyridine (10 ml) and Ac_2O (5 ml) and allowed to stand at room temperature for 24 h. Normal workup of the reaction mixture gave a mixture which was separated by circular preparative tlc over Si gel G (solvent, toluene-EtOAc-HOAc, 96:3:1) to give the monoacetate [**9**] (40 mg) and the diacetate [**10**] (15 mg).

Methyl 16β-hydroxy-17-acetoxy-(−)-kauran-19-oate [**9**].—Gum from $CHCl_3$; ir max 3450, 1720, 1700 cm^{-1}; ^{1}H nmr δ 0.83 (s, 3H, 20-CH_3), 1.16 (s, 3H, 18-CH_3), 2.11 (s, 3H, 17-OAc), 3.64 (s, 3H, OCH_3), 3.90, 4.04 (ABq, J=11 Hz, 2H, 17-CH_2); ^{13}C nmr see Table 2; eims (*m/z*, rel. int.) 392.2551 (M^+,7%) (calcd $C_{23}H_{36}O_5$ 392.2563), 374 (3%), 362 (3%), 319 (100%), 314 (17%), 289 (52%), 259 (30%), 255 (14%), 223 (12%), 123 (34%), 121 (42%), 109 (29%), 107 (33%).

Methyl 16β,17-diacetoxy-(−)-kauran-19-oate [**10**].—Oil from $CHCl_3$; ir max 1740, 1720, 1700 cm^{-1}; ^{1}H nmr δ 0.83 (s, 3H, 20-CH_3), 1.16 (s, 3H, 18-CH_3), 2.00, 2.11 (2×s, 2×3H, 2×OAc), 3.64 (s, 3H, OCH_3), 4.40 (s, 2H, 17-CH_2); ^{13}C nmr see Table 2; eims (*m/z*, re. int.) 434.2671 (M^+,3%) (calcd $C_{25}H_{38}O_6$ 434.2668, 374 (85%), 361 (62%), 344 (15%), 319 (77%), 314 (100%), 299 (16%), 289 (18%), 284 (13%), 259 (22%), 255 (35%), 123 (30%), 121 (53%), 109 (31%), 107 (29%).

Oxidation of **1** *to give methyl 16α,17-dihydroxy-(−)-kauran-19-oate* [**3**].—Compound **1** (200 mg) in Et_2O (10 ml) and pyridine (5 ml) was treated with OsO_4 (300 mg) at 0° for 24 h. The reaction mixture was diluted with Et_2O and

treated with a solution of mannitol (5 g) and KOH (5 g) in H_2O (50 ml). The mixture was refluxed for 2 h, and the organic layer was evaporated off to leave a residue which was washed with 5% HCl and H_2O. Column chromatography of the residue over Si gel 60 eluting with EtOAc yielded **11** (150 mg), which was recrystallized from Me_2CO as needles, mp 264-266° [lit. (9) 266-268°]. Treatment of **11** (50 mg) with CH_2N_2 gave **3** as needles from $CHCl_3$/MeOH, mp 152-154° [lit. (14) 153-154°]; ir max 3400, 1730 cm^{-1}; ^1H nmr δ 0.82 (s, 3H, 20-CH_3), 1.16 (s, 3H, 18-CH_3), 3.64 (s, 3H, OCH_3), 3.66, 3.78 (ABq, J=11 Hz, 2H, 17-CH_2); ^{13}C nmr see Table 2; eims (*m/z*, rel. int.) 350.2466 (M^+,2%) (calcd $C_{21}H_{34}O_4$ 350.2457), 319 (100%), 287 (14%), 277 (13%), 273 (34%), 259 (50%), 123 (46%), 121 (58%), 109 (45%), 107 (43%).

Mixture of methyl 17-hydroxy-16β-(−)-kauran-19-oate [**12**] *and methyl 17-hydroxy-16α-(−)-kauran-19-oate* [**13**].—Gum from $CHCl_3$; ir max 3400, 1720 cm^{-1}; ^1H nmr δ 0.81 (s, 3H, 20-CH_3), 1.16 (s, 3H, 18-CH_3), 3.64 (s, 3H, OCH_3), 3.40 (d, J=7 Hz, 1H, CH_2-17α), 3.70 (d, J=7 Hz, 1H, CH_2-17β); ^{13}C nmr see Table 2; eims (*m/z*, rel. int.) 334.2496 (M^+,54%) (calcd $C_{21}H_{34}O_3$ 334.2508), 275 (100%), 123 (51%), 109 (37%), 107 (22%). Compounds **12**/**13** (50 mg) in pyridine (10 ml) on treatment with Ac_2O (5 ml) at room temperature for 12 h gave a mixture of the corresponding 17-acetoxy derivatives; ^1H nmr δ 2.01 (s, 3H, 17-OAc), 3.85 (d, J=7 Hz, 1H, CH_2-17α), 4.15 (d, J=7 Hz, 1H, CH_2-17β); ^{13}C nmr see Table 2.

Mixture of 17-hydroxy-16β-(−)-kauran-19-oic acid [**16**] *and 17-hydroxy-16α-(−)-kauran-19-oic acid* [**17**].—Crystallized as cubes from $CHCl_3$; $[\alpha]^{25}D$ −130° (*c* 0.01, $CHCl_3$); ir max 3400, 1700 cm^{-1}; ^1H nmr δ 0.93 (s, 3H, 20-CH_3), 1.23 (s, 3H, 18-CH_3), 3.40 (d, J=7 Hz, 1H, CH_2-17α), 3.70 (d, J=7 Hz, 1H, CH_2-17β); eims (*m/z*, rel. int.) 320.2359 (M^+,95%) (calcd $C_{20}H_{32}O_3$ 320.2351), 289 (19%), 274 (68%), 243 (14%), 123 (100%), 121 (77%), 109 (97%), 107 (73%). Treatment of a mixture of **16** and **17** in Et_2O with CH_2N_2 gave a product identical in all respects to the mixture of **12** and **13**.

Caryophyllene-4,5-oxide.—Oil, $[\alpha]^{25}D$ −66° (*c* 0.01, $CHCl_3$) [lit. (18) 68°]; ir max 3060, 1620 (C=CH_2), 1260, 870 (epoxide) cm^{-1}; eims (*m/z*, rel. int.) 220.1839 (M^+,6%) (calcd $C_{15}H_{24}O$ 220.1827). Spectral data in agreement with that published (6).

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