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ENT-PIMARANES, *ENT*-KAURANES, HELIANGOLIDES AND OTHER CONSTITUENTS OF THREE *HELIANTHUS* SPECIES

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Key Word Index—*Helianthus hirsutus*, *H petiolaris*, *H strumosus*, Compositae, Heliantheae, diterpenes, *ent*-pimaranes, *ent*-kauranes, flavones, chromenes, heliangolides, sesquiterpene lactones

Abstract—Aerial parts of *Helianthus strumosus* gave the C-2′ epimer of the heliangolide 2′,3′-dihydrobudlein A as well as the flavones nevadensin, hymenoxin, sudachitin and acerosin, and three *ent*-pimaranes, two of which, *ent*-pimara-7,15-dien-19-oic acid and *ent*-7-oxopimara-8,15-dien-19-oic acid, are new *H petiolaris* furnished the heliangolide budlein A, ciliaric acid, and several *ent*-kauranoic acids, including the new *ent*-16β,17-dihydroxykauran-19-oic acid *H hirsutus* gave three known chromenes, hymenoxin, budlein A, two new diterpenes *ent*-8(*R*),15(*S*)-*epoxy*-12β-acetoxy-16-hydroxypimar-9(11)-en-19-oic acid and *ent*-12α-acetoxypimar-9(11)-en-19-oic acid and a new fatty acid derivative 9,16-dioxooctadec-10,12,14-trienoic acid

INTRODUCTION

In continuation of our earlier work on *Helianthus* species [1–7] we have examined *Helianthus hirsutus* Raf, *H petiolaris* Nutt and *H strumosus* L *H strumosus* and *H hirsutus* differed from *H petiolaris* and other *Helianthus* species studied previously in containing *ent*-pimarane-type diterpenes, several of them new, instead of *ent*-kauranoic and *ent*-trachylobanic acids which are commonly found in the genus [3, 5–7, 8–16] Each of the three species furnished a sesquiterpene lactone of the heliangolide type found in other representatives of the genus [1–6, 8, 9, 12, 14, 16–21] as well as other compounds

RESULTS AND DISCUSSION

H strumosus furnished besides the known flavones nevadensin (**6a**, previously found also in *H argophyllus* [12] and *H pumilus* [1]), hymenoxin (**6b**, previously found in *H angustifolius* [9, 22] and *H simulans* [6]), sudachitin (**6c**) and acerosin (**6d**), the known *ent*-pimara-8(14),15-dien-19-oic acid (**1a**) [23–25], isolated as its methyl ester **1b**, two new *ent*-pimaradienes **2a** and **4a**, also as their methyl esters, and a new heliangolide **7a**

The less polar new methyl ester, isolated in very small amount admixed with **1b**, was methyl *ent*-pimara-7,15-dien-19-oate (**2b**) on the basis of its spectral properties Chemical shift and appearance of the ring vinyl proton signal at δ 5 38 as a multiplet ($W_{1/2}$ = 11 Hz) obviously coupled to two vicinal protons were comparable with the H-7 signal of methyl oblongifolate (**3**) [26] and its analogues On the other hand, **2b** clearly differed from **3** in the chemical shifts of H-15, H-16a,b and H-17 indicating a difference in stereochemistry at C-13

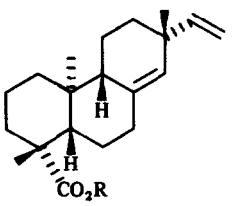
The more polar diterpene ester **4b** also had an NMR spectrum which suggested that it was a methyl pimarate The presence of an α,β-unsaturated ketone was indicated by the IR (strong band at 1652 cm^{-1}) and ^{13}C NMR spectrum (Table 1) The location of the carbonyl group at C-7 was discerned from the absence of additional vinylic proton signals and the presence of the typical AB system of H-6a and H-6b, each of which was vicinally coupled to H-5 Placement of the carbonyl group at C-7 was also accommodated by the ^{13}C NMR spectrum The absolute configuration of **4b** (and by implication that of **2b**) shown in the formulae is based on its CD curve which exhibited a

Table 1 ^{13}C NMR spectra of **4b**, **14a** and **15b** (67 89 MHz, $CDCl_3$)*

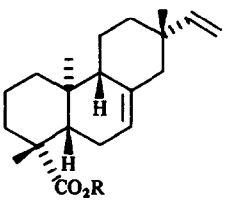
C	4b	14a	15b
1	37 29 *t*†	41 65 *t*	41 21 *t*
2	19 25 *t*	18 77 *t*	19 05 *t*
3	36 91 *t*	38 02 *t*	37 91 *t*
4	43 73	44 20	44 09
5	50 69 *d*	47 80 *d*	53 86 *d*
6	33 70 *t*‡	20 22 *t*	19 21 *t*
7	199 52	35 33 *t*	37 42 *t*†
8	129 17	29 04 *d*	76 74
9	163 50	157 31	146 72
10	40 02	38 81	38 72
11	22 71 *t*	115 58 *d*	124 94 *d*
12	32 94 *t*‡	73 52 *d*	77 67 *d*
13	34 02	38 44†	34 37
14	36 08 *t*†	27 15 *t*	37 82 *t*†
15	147 55 *d*	145 07 *d*	81 65 *d*
16	110 55 *t*	111 80 *t*	59 22 *t*
17	24 10 *q*	21 90 *q*‡	22 13 *q*
18	27 76 *q*	28 52 *q*	28 57 *q*
19	177 08	183 69	177 54†
20	16 10 *q*	21 81 *q*‡	21 17 *q*‡
OMe	51 45 *q*	—	51 30 *q*
OAc	—	21 36 *q*	20 83 *q*‡

*Unmarked signals are singlets

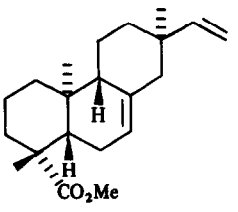
†‡Assignments with the same sign in each column may be interchanged



R
1a H
1b Me



R
2a H
2b Me



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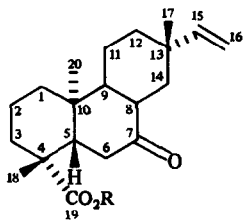
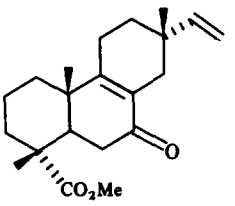


R
4a H
4b Me



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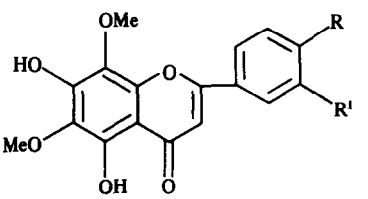
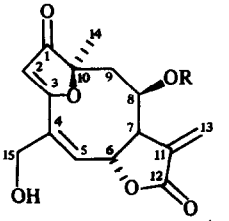
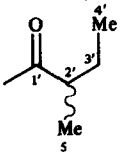
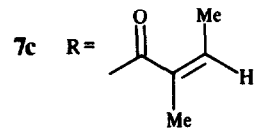


6a R=H, R¹=OMe
6b R, R¹=OMe
6c R=OMe, R¹=OH
6d R=OH, R¹=OMe

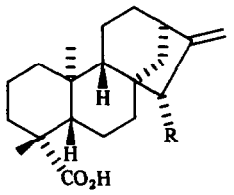


7a,b R=

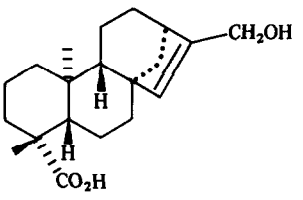




7c R=



R
8a H
8b OH



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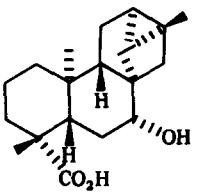


10

negative Cotton effect at 327 nm comparable in magnitude but opposite in sign to that of **5** of established absolute stereochemistry [27]

A $C_{20}H_{24}O_7$ sesquiterpene lactone (**7a**) isolated from *H strumosus*, mp 163–164°, had ^{1}H NMR and ^{13}C NMR signals essentially identical with those recently reported for 2',3'-dihydrobudlein A (**7b**), mp 180–181°, from *Viguiera hemsleyana* for which structure **7** (exclusive of the stereochemistry at C-2') has been deduced [28] Comparison of the ^{1}H NMR spectra run under identical conditions showed that the two compounds were not the same, small differences in the chemical shifts of several signals being observed (Table 2) As the coupling constants were identical, the two lactones had to be epimers at C-2' of the 2-methylbutanoate ester side chain An X-ray analysis of the new sesquiterpene lactone carried out by Dr John F Blount (Hoffmann-LaRoche) confirmed structure and stereochemistry of the sesquiterpene lactone moiety, unfortunately disorder in the five carbon ester side chain interfered with assignment of stereochemistry at C-2'

Extraction of a small collection of *H petiolaris* furnished the heliangolide budlein A (**7c**) previously isolated from *H angustifolius* [9] and several *Viguiera* species [28,

Table 2 ^{1}H NMR spectra of **7a** and **7b** (270 MHz, $CDCl_3$)

H	7a	7b
2	5 70	5 70
5	6 22 (*td*, 1 6, 4 3)	6 22
6	5 37 (*tt*, 1 6, 4 3)	5 39
7	3 75 (*dddd*, 4 3, 3, 3, 2)	3 72
8	5 26 (*ddd*, 5 5, 3, 2)	5 24
9a	2 47 (*dd*, 15, 5 5)	2 48
9b	2 27 (*m*)	2 27
13a	6 37 (*d*, 3)	6 37
13b	5 70 (*d*, 3)	5 70
14*	1 50 (*br*)	1 49
15†	4 37 (*t*, 1 6)	4 37
17	2 27 (*m*)	2 27
18†	1 58 (*sext*, 7)	1 58
19†	1 41 (*sext*, 7)	1 41
20*	0 85 (*t*, 7)	0 81
21*	1 07 (*d*, 7)	1 08

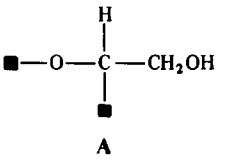
*Intensity three protons
†Intensity two protons

30, 31], ciliaric acid (**10**), a common constituent of *Helianthus* species [3, 5, 6, 12, 16, 20], and the *ent*-kaurenic acids **8a**, **8b**, **9**, **11a** and **11c** Diol **11a** appeared to be new, its ^{1}H NMR spectrum differed significantly from that of the known diol **11c** (in *H occidentalis*, *radula* and *simulans* [6, 7]) only in the frequencies of H-17a and H-17b (centre of AB system at δ 3 37 in **11a** vs δ 3 66 in **11c**) The structure was confirmed by periodic acid oxidation to **12**, previously obtained from **11c** and also naturally-occurring in *H radula* [7]

H hirsutus also gave budlein A (**7c**), hymenoxin (**6b**), three known chromenes **13a–c** (from *Helianthella uniflora* [32], *Helianthella quinquenervis* [33] and *Encelia californica* [34]), two new *ent*-pimarane derivatives **14a** and **15a**, and a new fatty acid constituent $C_{18}H_{26}O_4$ (high resolution MS) which was assigned structure **16** based on its UV spectrum (λ_{max} 316 nm), its easily interpretable ^{1}H NMR spectrum (see Experimental) and its mass spectral fragmentation (Scheme 1)

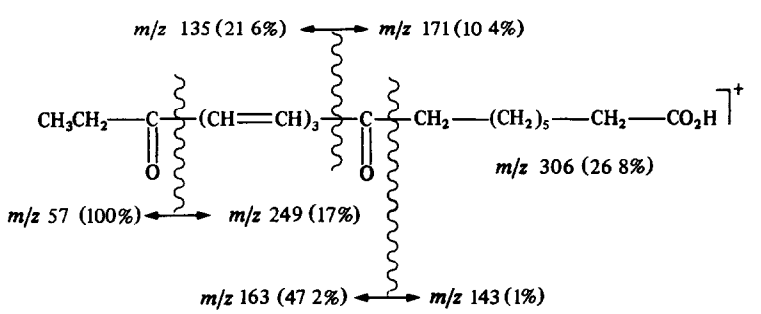
Structure **14a** for one of the two new pimaranes followed from the empirical formula, the IR spectra of the substance and its methyl ester and NMR spectrometry The ^{1}H NMR spectrum displayed three methyl singlets at δ 1 27, 1 02 and 0 99, the ABX system of the vinyl group attached to C-13 and a slightly broadened doublet at δ 4 94 The latter, presumably under an acetate (singlet at δ 1 98) because of its chemical shift, was coupled (J = 5 5 Hz) to the double doublet of a vinylic hydrogen at δ 5 33 which was in turn coupled allylically (J = 2 5 Hz) to a multiplet at δ 2 34 Because of the multiplicities, the protons responsible for these signals had to be located at C-12, C-11 and C-8 of the *ent*-pimarane skeleton The assignments were supported by the ^{13}C NMR spectrum (Table 1) which contained disubstituted and monosubstituted vinylic carbon signals at δ 157 31 and 115 58 (C-9 and C-11) and a doublet at 73 52 (C-12 carrying the acetate) On methylation to **14b**, the methyl singlet at δ 1 02 assigned to H-20 underwent a diamagnetic shift to δ 0 92, thereby establishing a diaxial relationship between the C-4 carboxyl and the C-10 methyl group β-Orientation of the acetate is based on the value of $J_{11,12}$ (5 5 Hz) and on the existence of long range coupling between H-12 and H-14b (model)

The ^{1}H NMR spectrum of a second new diterpene **15a**, isolated as its methyl ester **15b** of empirical formula $C_{23}H_{34}O_6$, contained three methyl singlets at δ 1 19, 1 00 and 0 88, signals of two mutually coupled (J = 2 5 Hz) protons at δ 5 68 (slightly broadened doublet, H-11) and δ 5 27 (sharp doublet, H-12), and an acetate at δ 2 12 There was also an ABX system whose X part at δ 3 62 was coupled to the AB part centred at δ 4 16 The latter shifted to δ 4 44 on conversion to monoacetate **15c**, an observation which revealed that the hydroxyl group indicated by the IR spectrum was primary and that the ABX system reflected the presence of partial formula **A** where ■ represents a quaternary carbon atom This assumption was also supported by the ^{13}C NMR spectrum (Table 1)

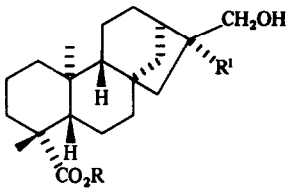


A

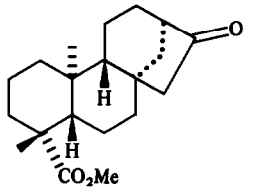
Because H-11 did not exhibit the allylic coupling observed in the NMR spectrum of **14a**, it was logical to assume that C-8 was tetrasubstituted and in fact was the point of attachment of the ether oxygen of partial structure **A** A plausible formula for the new diterpene acid was therefore **15a**, similar to the isodarutigenol analogues recently isolated from *Liatris laevigata* [35], but without commitment so far as to the stereochemistry



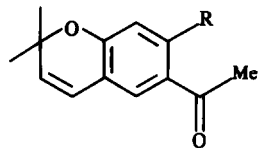
Scheme 1 Mass spectrum of **16**



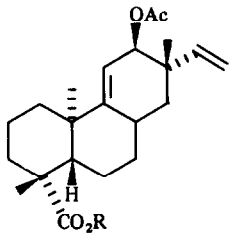
11a R = H, R^1 = β–OH
11b R = Me, R^1 = β–OH
11c R = H, R^1 = α–OH



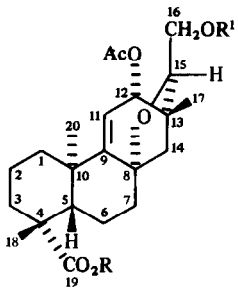
12



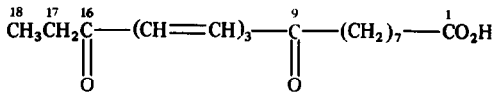
R
13a OH
13b H
13c OMe



R
14a H
14b Me



15a R, R^1 = H
15b R = Me, R^1 = H
15c R = Me, R^1 = Ac



16

at C-12, C-13 and C-15 This was elucidated as follows (1) The small coupling between H-11 and H-12 ($J_{11,12}$ = 2 5 Hz), and the lack of long range coupling between H-12 and H-14b, in contrast to the situation prevailing in **14a** where long range coupling was observed and $J_{11,12}$ = 5 5 Hz, indicated that the acetate on C-12 was α-orientated (model) (2) H-16a and H-16b were considerably deshielded ($\Delta\delta \sim 0$ 5) compared with H-16a and H-16b in isodarutigenol analogues lacking the α-orientated C-12 acetate [35] This could occur only if the oxygen bridge was also α-orientated (3) Conversion of **15b** to the acetate **15c** produced a paramagnetic shift (δ 0 08) of the H-20 resonance which was somewhat more pronounced (δ 0 11) on reaction of **15b** with trichloroacetylisocyanate (TAI) [36] This required orientation of C-16 as shown in the formula or *R*, if the absolute stereochemistry of the new pimaranes from *H hirsutus* was the same as that of all other diterpenes hitherto isolated from *Helianthus* species

In a recent revision [37] of Heiser's infrageneric classification of *Helianthus* [38], *H strumosus* and *H hirsutus* were placed in series Corona-solis of section Divaricati, whereas *H petiolaris* was placed in section Helianthus The occurrence of *ent*-pimarane diterpenes and absence of *ent*-kauranes in *H strumosus* and *H hirsutus* differentiates these two species not only from other members of series Corona-solis or from section Divaricati in general, but from all other *Helianthus* species which have so far been investigated where kauranoid and trachelobanoid diterpenes are the norm The results on sesquiterpene lactone content of members of series Corona-solis are also not uniform [5] All members of section Helianthus studied so far give *ent*-kauranes, trachylobanes and sesquiterpene lactones of various types [12, 17, 18, 20] except for *H debilis*, two subspecies of which were devoid of lactones [6, 15]

EXPERIMENTAL

Extraction of H strumosus Above ground parts of *H strumosus* (2 kg) collected by Dr B H Braun in summer 1959 in the vicinity of Kansas City, Mo., were extracted with $CHCl_3$ and worked up in the usual fashion [39] The crude gum (5 g) was adsorbed on 10 g of silicic acid (Mallinckrodt 100 mesh) and chromatographed over 200 g of the same adsorbent packed in hexane, 500 ml fractions being collected as follows Frs 1–2 (Hex), 3–5 (hexane–EtOAc, 9 1), 6–7 (hexane–EtOAc, 4 1), 8–9 (hexane–EtOAc, 3 2), 10–11 (hexane–EtOAc, 1 1), 12–13

Table 3 ^{13}C NMR spectra of **7a** and **7b** (67 89 MHz, $CDCl_3$)*

C	7a	7b†
1	205 09	205 32
2	104 63 *d*	104 71 *d*
3	182 38	182 90
4	138 07	138 44
5	134 39 *d*	133 69 *d*
6	75 08 *d*‡	75 29 *d*§
7	48 48 *d*	48 42 *d*
8	73 78 *d*‡	74 05 *d*§
9	41 88 *t*	42 01 *t*
10	87 61	87 60
11	135 80	136 43
12	168 66	168 71
13	123 89 *t*	123 82 *t*
14	20 99 *q*	21 11 *q*
15	62 47 *t*	62 11 *t*
16	174 93	175 03
17	41 00 *d*	40 95 *d*
18	26 21 *t*	26 47 *t*
19	11 42 *q*	11 37 *q*
20	16 30 *q*	16 02 *q*

*Unmarked signals are singlets

†Taken from ref [28]

‡Assignments made by selective decoupling

§Assignments interchanged from those given in ref [28]

(hexane–EtOAc, 2 3), 14–17 (hexane–EtOAc, 1 4), 18–21 (EtOAc), 22–25 (EtOAc–MeOH, 49 1), 26–27 (EtOAc–MeOH, 19 1), 28–29 (EtOAc–MeOH, 9 1)

Purification of fr 3 (22 mg) by TLC (C_6H_6–EtOAc, 39 1, two developments), methylation of the impure (by NMR criteria) product with CH_2N_2 followed by further TLC using the same solvent system afforded a 1 2 mixture (10 mg) of methyl *ent*-pimara-8(14),15-dien-19-oate (**1b**) and methyl *ent*-pimara-7,15-dien-19-oate (**2b**), ^{1}H NMR of **1b** (270 MHz, $CDCl_3$) δ 5 72 (*dd*, J = 17, 10 5 Hz, H-15), 5 15 (*m*, $W_{1/2}$ = 5 Hz, H-14), 5 0–4 87 (*m*, H-16a, b), 3 63 (OMe), 1 20 (H-18), 1 00 (H-17), 0 56 (H-20), ^{1}H NMR of **2b** δ 5 70 (*dd*, J = 17, 10 5 Hz, H-15), 6 38 (*m*, $W_{1/2}$ = 11 Hz, H-7), 5 0–4 87 (*m*, H-16a, b), 3 65 (OMe), 1 19 (H-18), 0 97 (H-17) and 0 63 (H-20)

Methylation of fr 12 and purification by TLC (C_6H_6–EtOAc, 39 1, two developments) afforded 16 mg of methyl *ent*-7-oxopimara-8,15-dien-19-oate (**4b**) as a gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1720, 1657 and 1615, CD curve (MeOH) $[\theta]_{327}$ −3560, ^{1}H NMR (270 MHz, $CDCl_3$) δ 5 79 (*dd*, J = 17, 10 5 Hz, H-15), 4 93 (*dd*, J = 17, 1 Hz, H-16a), 4 91 (*dd*, J = 10 5, 1 Hz, H-16b), 3 68 (OMe), 2 94 (*dd*, J = 18, 14 Hz, H-6a), 2 74 (*dd*, J = 18, 4 Hz, H-6b), 1 21 (*br*, H-18), 0 94, 0 93 (*br*, H-17 and H-20), ^{13}C NMR Table 1 [Calc for $C_{21}H_{30}O_3$ MW, 330 2194 Found MW (MS), 330 2163] Other significant peaks in the HRMS were at *m/z* (rel int) 315 $[C_{20}H_{27}O_3]^+$ (31), 289 $[C_{18}H_{25}O_3]^+$ (100), 255 $[C_{18}H_{23}O]^+$ (42), 230 $[C_{16}H_{22}O]^+$ (4), 230 $[C_{16}H_{22}O]^+$ (4) and 229 $[C_{16}H_{21}O]^+$ (62)

Fr 17 on purification by TLC ($CHCl_3$–MeOH–EtOAc, 18 1 1) gave 12 mg of nevadensin (**6a**), mp 183–185°, identical with authentic material Frs 21 and 22 on standing in hexane–EtOAc deposited 20 mg of hymenoxin (**6b**), mp 214–217°, identical with authentic material Purification of fr 23 by TLC ($CHCl_3$–MeOH–EtOAc, 18 1 1, two developments) and recrystallization from $CHCl_3$–MeOH gave 6 mg of a 3 1 mixture of sudachitin (**6c**) and acerosin (**6d**), mp 223–225°, identified by comparison of the NMR spectrum with spectra of authentic samples Fr 25 had one main constituent, purification by TLC ($CHCl_3$–MeOH–EtOAc, 8 1 1) furnished 73 mg of **7a**, mp 163–164° (EtOAc) whose ^{1}H and ^{13}C NMR spectra are listed in Tables 2 and 3 [Calc for $C_{20}H_{24}O_7$ MW, 276 1522 Found MW(MS), 376 1525] Significant peaks in the low resolution MS were at *m/z* (rel int) 376 $[M]^+$ (25 8), 292 (31 8), 292 (31 8), 274 (83 4), 248 (100), 246 (26 1), 231 (21 4), 230 (12 6), 228 (13 5), 204 (16 2), 203 (13 7), 188 (11 2), 187 (15 1), 159 (11 8), 152 (17 1), 138 (63 1), 121 (20 8), 109 (15 9), 93 (10 8), 91 (20 5) and 85 (99 0)

Extraction of H petiolaris Above ground parts of *H petiolaris* (0 75 kg), collected by Dr B H Braun in summer 1959 in eastern Kansas, were extracted with $CHCl_3$ and worked up in the usual fashion The crude gum (3 5 g) was adsorbed on 5 g of silicic acid and chromatographed over 200 g of the same adsorbent packed in hexane, 125 ml fractions being collected as follows 1–4 (hexane), 5–8 (hexane–EtOAc, 9 1), 9–12 (hexane–EtOAc, 4 1), 13–16 (hexane–EtOAc, 3 2), 17–20 (hexane–EtOAc, 2 3), 21–24 (hexane–EtOAc, 1 4), 25–28 (EtOAc), 29–32 (EtOAc–MeOH, 9 1)

Fr 6 on purification by TLC (C_6H_6–EtOAc, 39 1) gave 34 mg of **8a**, mp 176–178° Frs 9 and 10 on standing in hexane–EtOAc deposited crystals which were identified as a 1 1 mixture of grandiflonic acid (**8b**) and **9** by NMR spectrometry Trituration of frs 12 and 18 with hexane–EtOAc furnished 15 mg of ciliaric acid (**10**), mp 290°, and 5 mg of **11a**, respectively Diol **11a** melted at 296–300° (MeOH) IR ν_{max}^{KBr} cm^{-1} 3400–2500 (very broad), 1700, ^{1}H NMR (270 MHz, $CDCl_3$ and 3 drops DMSO-d_6) δ 3 43 (*d*, J = 11 Hz, H-17a), 3 32 (*d*, J = Hz, H-17b), 1 10 (*br*, H-18), 0.98 (*br*, H-20) The low resolution MS did not exhibit the molecular ion, but had significant peaks at *m/z* (rel int) 318 $[M - H_2O]^+$ (0 6), 305 (100), 287 (34), 259 (54) and 241 (16) The material from the mother liquor of fr 18 was methylated with CH_2N_2 and purified by TLC (C_6H_6–EtOAc, 9 1) to yield 9 mg of **11b**, ^{1}H NMR (270 MHz, $CDCl_3$) δ 3 48 and 3 39 (*d*, J = 11 Hz, H-17a,b), 1 17 (*br*, H-18), 0 85 (*br*, H-20) A soln of 8 mg of **11b** in 1 5 ml of MeOH was stirred with 15 mg of periodic acid at room temp for 1 hr Purification of the product after the usual work-up by TLC (C_6H_6–EtOAc, 19 1) furnished 3 mg of **12**, identical with material previously [7, 40] obtained by degradation of **11c**

Fr 24 had one main constituent, purification by TLC ($CHCl_3$–MeOH–EtOAc, 8 1 1) gave 12 mg of **7c** as a gum Fr 26 on trituration with EtOAc afforded 15 mg of **11c**, mp 265–267° (MeOH)

Extraction of H hirsutus Above ground parts of *H hirsutus* Raf (1 4 kg), collected by Dr B H Braun in summer 1959 in the Kansas City area, were extracted with $CHCl_3$ and worked up in the usual fashion The crude gum (9 g) was adsorbed on 15 g of silicic acid and chromatographed over 250 g of the same adsorbent packed in hexane, 250 ml fractions being collected as follows Frs 1–2 (hexane), 3–6 (hexane–EtOAc, 19 1), 7–10 (hexane–EtOAc, 9 1), 11–14 (hexane–EtOAc, 4 1), 15–18 (hexane–EtOAc, 2 1), 19–22 (hexane–EtOAc, 1 1), 23–26 (hexane–EtOAc, 2 3), 27–30 (hexane–EtOAc, 1 4), 31–34 (EtOAc), 35–36 (EtOAc–MeOH, 49 1), 37–40 (EtOAc–MeOH, 19 1), 41–44 (EtOAc–MeOH, 9 1)

Purification of fr 4 by TLC (C_6H_6–EtOAc, 39 1) gave **13a** (39 mg), mp 79–81° (hexane) Similar treatment of fr 5 gave **13c** (4 mg) as a gum Repeated purification of fr 11 by TLC (C_6H_6–EtOAc, 9 1) and recrystallization (Et_2O–hexane) gave 41 mg of *ent*-12α-acetoxypimar-9(11),15-dien-19-oic acid (**14a**), mp 123–124°, $[\alpha]_D$ +106° (*c* 0 28 g/100 ml, $CHCl_3$), ^{1}H NMR (270 MHz, $CDCl_3$) δ 5 82 (*dd*, J = 16, 11 Hz, H-15), 5 53 (*dd*, J

= 5 5, 2 5 Hz, H-11), 4 99 (*dd*, *J* = 16, 1 Hz, H-16a), 4 98 (*dd*, *J* = 11, 1 Hz, H-16b), 4 94 (*d* (*br*), *J* = 5 5 Hz, H-12), 2 34 (*m*, H-8), 1 98 (Ac), 1 27 (*br*, H-18), 1 02 (*br*, H-20), 0 99 (*br*, H-17), ^{13}C NMR Table 1 [Calc for $C_{22}H_{32}O_4$ MW, 360 2300 Found MW(MS), 360 2312] Significant peaks in the low resolution MS were at *m/z* (rel int) 360 $[M]^+$ (0 3), 318 (0 6), 300 (8 8), 292 (45 2), 285 (2 9), 250 (100), 235 (3 2), 206 (3 0), 204 (2 1), 189 (2 2), 161 (2 2), 159 (2 1), 157 (1 8), 147 (4 2), 145 (4 3), 133 (5 9), 131 (6 5), 105 (8 8), 95 (24 7)

Fr 12 which showed many spots on TLC was methylated with CH_2N_2 Purification by TLC (C_6H_6–EtOAc, 19 1, two developments) gave 27 mg of **14b**, mp 116–117° ($CHCl_3$–MeOH), IR ν_{max}^{KBr} cm^{-1} 1730, 1635, ^{1}H NMR (270 MHz, $CDCl_3$) δ 5 82 (*dd*, *J* = 16, 11 Hz, H-15), 5 53 (*dd*, *J* = 5 5, 2 5 Hz, H-11), 4 99 (*dd*, *J* = 16, 1 Hz, H-16a), 4 98 (*dd*, *J* = 11, 1 Hz, H-16b), 4 94 (*d* (*br*), *J* = 5 5 Hz, H-12), 3 65 (OMe), 2 33 (*m*, H-8), 1 97 (Ac), 1 20 (*br*, H-18), 0 98 (*br*, H-17), 0 92 (*br*, H-20) [Calc for $C_{23}H_{34}O_4$ MW, 347 2457 Found MW(MS), 374 2469] Significant peaks in the low resolution MS were at *m/z* (rel int) 374 $[M]^+$ (0 9), 359 (0 8), 332 (1 1), 315 (21 0), 214 (25 6), 306 (82 5), 299 (4 2), 264 (100), 255 (4 4), 249 (3 6), 239 (3 2), 220 (2 6), 204 (6 0), 187 (4 5), 169 (7 1), 147 (7 4), 105 (11 4), 96 (23 7)

Fr 21 on standing in hexane–EtOAc deposited 4 mg of 9,16-dioxo-octadec-10,12,14-trienoic acid (**16**), mp 154–155° ($CHCl_3$), UV λ_{max}^{MeOH} 316 nm, IR ν_{max}^{KBr} cm^{-1} 3600–2400 (broad), 1700, 1685 and 1600, ^{1}H NMR (270 MHz, $CDCl_3$) δ 7 21 (*m*, H-11, 14), 6 68 (*m*, H-12, 13), 6 33 and 6 31 (each *d*, *J* = 16 Hz, H-10, 15), 2 61 (*q*, *J* = 7 Hz, H-17a, b), 2 56 (*t*, *J* = 7 Hz, H-8a, b), 2 35 (*t*, *J* = 7 Hz, H-2a,b), 1 64 (*q* (*br*), *J* = 7 Hz, H-3a,b, H-7a,b), 1 34 (*br*, H-3a,b, H-4a,b, H-6a,b), 1 13 (*t*, *J* = 7 Hz, H-18) [Calc for $C_{18}H_{26}O_4$ MW, 306 1831 Found MW(MS), 306 1855] Other significant peaks in the HRMS were at *m/z* (rel int) 249 $[C_{15}H_{21}O_3]^+$ (15), 178 $[C_{11}H_{14}O_2]^+$ (39), 171 $[C_9H_{15}O_3]^+$ (9), 163 $[C_{10}H_{11}O_2]^+$ (53) and 135 $[C_9H_{11}O]^+$ (21)

The residue from the mother liquors of fr 21 was purified by TLC ($CHCl_3$–MeOH–EtOAc, 18 1 1) The upper band yielded 3 mg of hymenoxin (**6b**) The lower band was not homogeneous, methylation with CH_2N_2 followed by TLC (C_6H_6–EtOAc, 4 1, two developments) furnished 19 mg of methyl *ent*-8(*R*),15(*S*)-epoxy-12β-acetoxy-16-hydroxypimar-9(11)-en-19-oate (**15b**) (gum), $[\alpha]_D$ + 143° (*c* 0 38 g/100 ml, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3530, 1720 (broad), ^{1}H NMR (270 MHz, $CDCl_3$) δ 5 68 (*d* (*br*), *J* = 2 5 Hz, H-11), 5 27 (*d*, *J* = 2 5 Hz, H-12), 4 21 (*dd* (*br*), *J* = 9 5, 3 Hz, H-16a), 4 11 (*dd*, *J* = 11, 9 5 Hz, H-16b), 3 64 (OMe), 3 62 (part obsc, H-15), 2 12 (Ac), 1 73 (*d*, *J* = 14 Hz, H-14a), 1 43 (*d* (*br*), *J* = 14 Hz, H-14b), 1 19 (*br*, H-18), 1 00 (*br*, H-17), 0 88 (*br*, H-20), ^{1}H NMR (C_6D_6) δ 5 80 (H-11), 5 33 (H-12), 4 13 (centre of AB system, H-16a, b), 3 50 (*dd* (*br*), *J* = 11, 3 Hz, H-15), 3 30 (OMe), 1 66 (Ac), 1 34 (H-14a), 0 94 (H-14b), 1 27, 1 11, 0 58 (H-18, H-17 and H-20), NMR ($CDCl_3$ + TAI) δ 8 56 (*br*, NH), 5 68 (H-11), 5 31 (H-12), 4 64 (centre of AB system, H-16a,b), 4 44 (*dd*, *J* = 11, 3 Hz, H-15), 3 63 (OMe), 2 14 (Ac), 1 20 (H-18), 1 00 and 0 99 (H-17, H-20), ^{13}C NMR Table 1 [Calc for $C_{23}H_{34}O_6$ MW, 406 2355 Found MW(MS), 406 2343] Significant peaks in the low resolution MS were at *m/z* (rel int) 407 1 $[M+1]^+$ (1 2), 406 $[M]^+$ (0 2), 391 (0 8), 379 (1 7), 373 (1 8), 363 (6 9), 362 (4 4), 358 (1 9), 345 (10 6), 331 (7 3), 329 (3 3), 313 (5 6), 303 (11 5), 302 (17 5), 289 (11 5), 287 (15 5), 287 (15 5), 285 (23 5), 271 (26 8), 243 (23 4), 227 (28 0), 225 (23 6), 211 (29 6), 203 (27 9), 173 (29 1), 169 (38 1), 161 (24 1), 159 (24 6), 147 (28 2), 145 (28 6), 125 (62 3), 121 (100)

Acetylation of 8 mg of **15b** with 0 2 ml Ac_2O in 0 5 ml pyridine overnight, work-up in the usual manner and purification by TLC (C_6H_6–EtOAc, 4 1), gave **15c** (6 mg), mp 197–198° ($CHCl_3$–MeOH), ^{1}H NMR (270 MHz, $CDCl_3$) δ 5 66 (*d* (*br*), *J* = 2 5, H-11), 5 29 (*d*, *J* = 2 5 Hz, H-12), 4 44 (centre of AB system, H-16a, b), 4 34 (obsc by H-16b, H-15), 3 62 (OMe), 2 12 and 2 11 (Ac), 1 66 (*d*, *J* = 14 Hz, H-14a), 1 47 (*d* (*br*), *J* = 14 Hz, H-14b), 1 19 (*br*, H-18), 1 00 (*br*, H-17), 0 97 (*br*, H-20) [Calc for $C_{25}H_{36}O_7$ MW, 448 2461 Found MW(MS), 448 2447] Significant peaks in the low resolution MS were at *m/z* (rel int) 449 $[M+1]^+$ (0 2), 448 $[M]^+$ (0 07), 405 (2 1), 388 (0 7), 387 (1 0), 362 (1 1), 345 (6 3), 331 (2 5), 329 (4 3), 328 (4 6), 319 (4 3), 315 (4 3), 313 (3 1), 302 (21 7), 289 (9 3), 287 (14 0), 285 (15 7), 271 (21 9), 243 (22 6), 241 (17 6), 227 (30 0), 225 (24 5), 211 (32 3), 203 (18 4), 173 (31 3), 161 (24 3), 159 (27 3), 147 (27 4), 145 (25 6), 135 (60 2), 121 (100) Fr 26 on purification by ($CHCl_3$–MeOH–EtOAc, 8 1 1) gave 34 mg of **7c** which could not be induced to crystallize

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