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SESQUITERPENE LACTONES FROM *CENTAUREA REPENS*

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Key Word Index—*Centaurea repens*; Compositae; Russian knapweed; allelopathy; sesquiterpenes; cynaropicrin; repin; janerin; repdiolide.

Abstract—Six sesquiterpene lactones have been isolated and characterized from *Centaurea repens*. They are identified as cynaropicrin, repin, janerin, aguerin B, repdiolide and epoxyrepdiolide.

INTRODUCTION

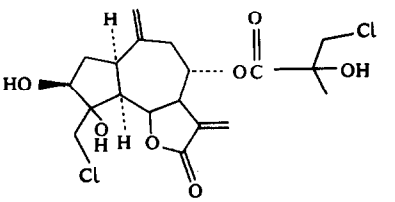
Centaurea species (Compositae) have been reported to contain a wide variety of sesquiterpene lactones, many of which have been shown to be biologically active [1–8]. The wide range of activity associated with this class of compounds, e.g., cytotoxicity, phytotoxicity, antineoplasticity, allergenicity etc. [9], suggested the possibility of allelopathy playing a major role in the invasiveness of Russian knapweed (*Centaurea repens* L.). Russian knapweed, referred to as *Centaurea picris* or *Acroptilon repens* in some literature [1], is a perennial herb which is rapidly becoming a major threat in many parts of the United States. The apparent ease with which this weed, and other related species, invade established range and croplands prompted us to investigate the chemical make-up of the plant in an attempt to isolate and identify phytotoxic substances.

In addition to its suspected allelopathic activity, *C. repens* has been implicated [10] in the nervous disease in horses called equine nigropallidal encephalomalacia (ENE), a disorder caused by the necrosis and softening of specific brain tissue. The specific toxin has not been identified; however, the presence of sesquiterpene-α-methylene-γ-butyrolactones in *Centaurea* species and the known cytotoxicity of these lactones [7, 8] led us to suspect similar toxic substances in Russian knapweed as the causative agent(s) in ENE.

C. repens has been the subject of previous investigations in which the sesquiterpenes centaurepensin (chlorohyssopifolin A) (**1**) [3, 5], acroptilin (chlorohyssopifolin C (**2**) [2, 5] and repin (**4b**) [1] were isolated and characterized. Centaurepensin (**1**), acroptilin (**2**) and repin (**4b**) have all been inter-related [5] and in turn have been related to a derivative of cynaropicrin (**3b**) [11]. The structure and absolute stereochemistry have been well delineated by correlation with α-santonin [12]. However, the relative stereochemistry about carbons 4, 15, 17 and 18 in compounds **1**, **2** and **4b** are less certain.

RESULTS AND DISCUSSION

Sequential extraction of the dried, ground plant (aerial part) with Skellysolve-F, followed by ether,



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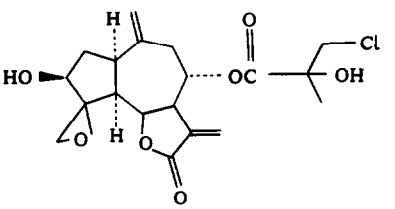

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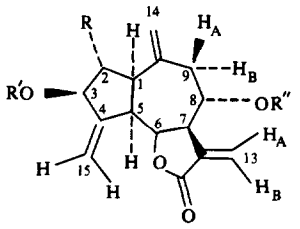
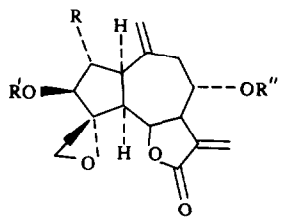
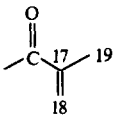
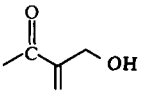
afforded six sesquiterpene lactones from the latter extract. Cynaropicrin (**3b**) (0.04%) was isolated by repeated chromatography on Si gel and identified by ^{1}H NMR, ^{13}C NMR, IR, mass spectrometry and optical rotation. This is the first report of cynaropicrin in *C. repens*. Further fractionation of the ether extract led to the isolation of repin (**4b**), janerin (**4c**) and aguerin B (**3c**) [1, 6, 13]. The TLC R_f values for each compound with three solvent systems are listed in Table 1. The structure and stereochemistry of aguerin B (**3c**) have been firmly established by conversion to a compound of known structure [6]. However, the relative stereochemistry of the 4,15-epoxide function in both repin and janerin was uncertain despite a report by Evstratova *et al.* [1] that repin contains a β-epoxide moiety. After extensive NMR studies, these authors incorrectly assigned a *trans* ring junction at C-1, C-5 [1, 5]. Hence, their stereochemical conclusion concerning the 4β,15-epoxide, which was based on the same NMR study, required further examination.

The ^{1}H NMR spectral data of repin (**4b**), janerin (**4c**) and aguerin B (**3c**) are shown in Table 2. The close similarity between repin and janerin, with the excep-

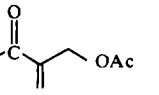


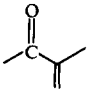


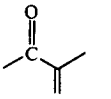
3a R = OH ; R' = H ; R'' = 

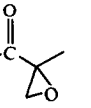
3b R = R' = H ; R'' = 

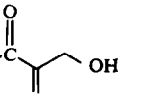
3c R = R' = H ; R'' = 

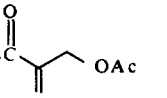
3d R = H ; R' = Ac ; R'' = 

3e R = OAc ; R' = Ac ; R'' = 

4a R = OH ; R' = H ; R'' = 

4b R = R' = H ; R'' = 

4c R = R' = H ; R'' = 

4d R = R' = Ac ; R'' = 

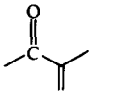
4e R = OAc ; R' = Ac ; R'' = 

Table 1. TLC R_f values for sesquiterpene lactones on Si gel

	EtOAc	Benzene–acetone (1:1)	Chloroform–methanol (9:1)
Aguerin B (3c)	0.50	0.53	0.56
Repin (4b)	0.43	0.49	0.55
Cynaropicrin (3b)	0.37	0.42	0.38
Janerin (4c)	0.34	0.40	0.40
Repdiolide (3a)	0.26	0.32	0.34
Epoxyrepdiolide (4a)	0.23	0.30	0.30

tion of the signals for H-18 and H-19, is striking and suggests that both compounds possess the same relative configuration around the 4,15-epoxide. Mompon and Toubiana [14] have characterized subluteolide as **4b** except for having a 4α,15-epoxide function. Comparison of the ^{1}H NMR spectrum of subluteolide (Table 2) with repin and janerin strongly suggests that these two compounds also have a 4α,15 epoxide function. Because of the known shielding effects of the oxirane ring [15], one would expect significant differences in the signals for H-1 and H-5, relative to subluteolide, if the epoxide ring were β-orientated. In addition, considerable shifts would be expected for the H-15 protons due to the effects of the C-3 hydroxyl and the lactone moieties. To confirm the stereochemistry at C-4, cynaropicrin (**3b**) was acetyl-

Table 2. ^{1}H NMR spectral data (90 MHz) of sesquiterpene lactones

	H-1	H-2	H-3	H-5	H-6	H-7	H-8	H-9A	H-9B	H-13A	H-13B	H-14	H-15A	H-15B	H-18A	H-18B	H-19	Ac
4b	3.37	1.80	3.96	2.02	4.61	3.03	5.07	2.33	2.70	5.53	6.19	4.94–	3.28	3.03	3.13	2.80	1.61	
($CDCl_3$)*	*dd*	*ddd*	*br*	*dd*	*dd*	*ddt*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.17	*br*	*br*	*d*	*d*	*s*	—
		2.43	*dd*									*br*						
		ddd										*m*						
4c	3.36	1.80	3.98	2.04	4.62	3.07	5.10	2.37	2.78	5.58	6.14	4.93–	3.28	3.06	6.30	5.94	4.35	—
($CDCl_3$)	*dd*	*ddd*	*dd*	*dd*	*dd*	*ddt*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.16	*br*	*br*	*s*	*s*	*s*	
		2.47										*br*						
		ddd																
3c	2.84	1.77	4.53	2.84	4.22	3.14	5.03	2.33	2.70	5.57	6.19	4.90–	5.33	5.47	6.16	5.63	1.94	
($CDCl_3$)	*dd*	*ddd*	*ddt*	*m*	*dd*	*ddt*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.10	*d*	*d*	*t*	*t*	*t*	—
		2.12										*dd*						
		ddd																
Subluteolide	3.40	1.86	4.00	2.08	4.62	3.12	5.04	2.34	2.80	5.72	6.25	4.96–	3.08	3.34	3.20	2.84	1.64	
	dd	*ddd*	*dd*	*dd*	*dd*	*m*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.19	*d*	*d*	*d*	*d*	*s*	—
		2.48										*d*						
		ddd																
3a	3.08	4.31	4.75	3.10	4.48	3.30	5.28	2.48	2.80	5.80	6.20	5.07–	5.61	5.61	6.22	5.80	1.96	—
(pyridine-d_5)	*dd*	*dd*	*ddd*	*dd*	*dd*	*ddt*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.32	*m*	*m*	*m*	*m*	*t*	
												d						
3a	2.83	3.80	4.23	2.83	4.23	3.16	5.03	2.47	2.67	5.60	6.17	4.97–	5.60	5.38	6.17	5.60	1.97	—
($CDCl_3$)	*dd*	*dd*	*ddd*	*dd*	*dd*	*ddt*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.17	*m*	*m*	*m*	*m*	*t*	
												dd						
3e	2.93	5.3	5.6	2.93	4.19	3.12	5.1	2.39	2.67	5.62	6.19	4.191–	5.62	5.27	6.12	5.62	1.93	1.97,
($CDCl_3$)	*dd*			*dd*	*dd*	*ddt*		*dd*	*dd*	*dd*	*dd*	5.08	*m*	*m*	*m*	*m*	*t*	2.07
												dd						*s*
4a	3.29	4.5	4.5	2.67	4.67	3.35	5.27	2.50	2.90	5.56	6.23	5.07–	3.44	3.54	6.17	5.58	1.99	—
(pyridine-d_5)	*dd*			*dd*	*dd*	*ddt*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.33	*m*	*m*	*m*	*m*	*t*	
												d						
4e	3.07	5.10	5.43	2.23	4.43	3.33	5.17	2.46	2.77	5.63	6.23	4.99–	3.33	2.96	6.16	5.63	1.96	2.00,
($CDCl_3$)	*dd*	*dd*	*ddd*	*dd*	*dd*	*ddt*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.20	*d*	*d*	*m*	*m*	*t*	2.06
												d						*s*
6	2.70	5.27	3.82	2.33	4.60	3.16	5.08	2.63	2.73	5.62	6.20	5.11–	4.29	4.16	6.13	5.60	1.97	2.04,
($CDCl_3$)	*dd*	*dd*	*d*	*dd*	*dd*	*ddt*	*ddd*	*dd*	*dd*	*dd*	*dd*	5.02	*d*	*d*	*m*	*m*	*t*	2.08
												d						*s*

*Solvents are given in parentheses.

J(Hz): 1, 2 = 8.8; 1, 5 = 11; 2, 3 = 8.1; 3, 15 = 2.4; 5, 6 = 10.0; 6, 7 = 9.0; 7, 8 = 9.9; 7, 13 = 3.6; 8, 9A = 2.7; 8, 9B = 5.1; 9A, 9B = 14.7; 13A, 13B = 0.6; 14, 14′ = 2.1; 18, 19 = 1.2. For **4a** and **4b**: 15A, 15B = 5. For **6**: 15A, 15B = 12.

ated then epoxidized with one equivalent of *m*-chloroperbenzoic acid at 0° to give 4,15-epoxycynaropicrin diacetate identical (IR, ^{1}H NMR, ORD) with janerin diacetate. Epoxidation of allylic and homoallylic acetates give *trans*-epoxides, relative to the acetate [16], hence janerin and repin have a 4α,15-epoxide function.

From the spectral data, it is apparent that subluteolide and repin differ in configuration at C-17. This is indicated by the pronounced difference in the chemical shift of H-9B (δ2.70 vs 2.80), H-13A (δ5.53 vs 5.72) and H-7 (δ3.03 vs 3.12) all of which are projected toward C-17 and would be expected to be affected by changes at C-17.

Further chromatography afforded a new lactone, repdiolide (**3a**), as an oil with a molecular formula of $C_{19}H_{22}O_6$, MW (MS) 346. Loss of *m/z* 86 (α-methyl acrylic acid) is consistent with an α-methyl acrylate side chain. The IR spectrum shows strong hydroxyl bands at 3420 cm^{-1} and carbonyl bands at 1760 cm^{-1} (characteristic of α-methylene-γ-lactones) and 1720 cm^{-1} (ester).

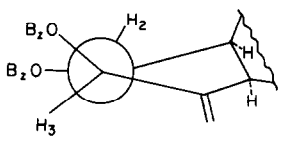
The ^{1}H NMR spectra of **3a** in chloroform and pyridine-d_5 is shown in Table 2. Coupling constants and assignments have been deduced via extensive decoupling experiments and use of europium shift reagent. A vinyl methyl group at δ 1.96 (*t*, J = 1.2 Hz) and vinyl protons at 6.22 (*br*) and 5.80 (*br*) confirm the presence of the α-methyl acrylate side chain. The exocyclic methylene groups have resonances at 5.80 (*dd*, J = 0.6, 3.6 Hz, H-13A), 6.20 (*dd*, J = 0.6, 3.6 Hz, H-13B), 5.07 and 5.32 (pair of doublets, J = 2.1 Hz, H-14), 5.61 (*m*, H-15A and H-15B), consistent with numerous other sesquiterpenes possessing similar groups [1, 2, 5, 13].

Two carbinol protons (H-2 and H-3) occur at 4.31 (*dd*, J = 8.1, 8.8 Hz) and 4.75 (*ddd*, J = 8.1, 2.4, 2.4 Hz) which shift to 5.3 and 5.6 on acetylation to form repdiolide diacetate (**3e**). The additional coupling (2.4 Hz) of the proton resonating at 4.75 arises from H-15A and H-15B, consistent with other guianolides possessing a 3-hydroxyl group, e.g. cynaropicrin (**3b**) has $J_{3,15}$ = 1.8 Hz. Protons at C-6, C-7 and C-8 occur at 4.48, 3.30 and 5.28 respectively with large (9–9.9 Hz) coupling constants suggesting an all-*trans*-configuration around these centers. Likewise, the large (10 Hz) coupling between H-5 and H-6 indicates a *trans*-configuration. Other guianolides having the same relative configuration around these centres have similar ^{1}H NMR spectra [1, 5, 17].

The C-9 methylene group shows a pair of doublets with that for H-9A occurring at δ2.48 (*dd*, J = 14.7, 2.7 Hz) and that for H-9B at 2.80 (*dd*, J = 14.7, 5.1 Hz). To determine the nature of the ring junction around C-1 and C-5, H-9A was irradiated while monitoring the intensity of the signal for H-6. A 14% increase in the intensity was observed. Herz [18] has shown that a close proximity of H-6 and H-9A necessitates a *cis*-ring junction, hence the large NOE observed indicates a *cis*-fusion. This was confirmed by observation of the large coupling constant (J = 11 Hz) between H-1 and H-5, again consistent with other sesquiterpene lactones of similar geometry.

Although $J_{1,2}$ and $J_{2,3}$ are known (8.8 and 8.1 Hz respectively) the relative configuration of the vicinal diol cannot be determined on the basis of the ^{1}H NMR data alone. Application of the Karplus equation is risky at best and must be used with caution when applied to five-membered rings for which few models exist. That the hydroxyl groups are *trans*, i.e. 2α, 3β, is based on the facts that: (1) the compound would not form an acetonide nor a thioacetonide, (2) no carbonate could be formed with phosgene, (3) acetylation with one equivalent of acetic anhydride gave the C-2 acetate, the less hindered position only if the C-2 hydroxyl is α and (4) no NOE was observed between H-2 and H-3 in the monoacetate [14].

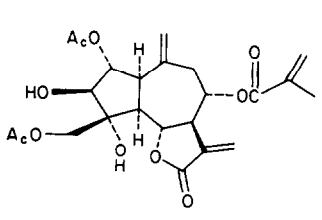
Treatment of repdiolide (**3a**) with benzoyl chloride in pyridine afforded the dibenzoate (**5**) as an oil which showed a CD spectrum with a strong negative $\Delta\epsilon$ at 238 nm and a positive $\Delta\epsilon$ at 233 nm. Application of the Exciton Chirality method of Harada and Nakanishi [19, 20], showed that the benzoate functions must have the configuration as depicted in **5**, i.e. a configuration leading to a negative chirality. Since the diol has been established to be *trans*, the negative chirality in the CD spectrum of **5** establishes the absolute configuration of repdiolide as depicted in **3a**.



5

The third sesquiterpene lactone isolated, mp 129–136°, was 4,15-epoxyrepdiolide (**4a**), $C_{19}H_{22}O_7$ (*m/z* 362). The mass spectrum showed a prominent ion at *m/z* 276 ([M − 86]$^+$, α-methyl acrylic acid) thus showing the same side chain as repdiolide. An epoxide is indicated by a strong ion at *m/z* 346 [M − 16]$^+$, which was confirmed by an examination of its ^{1}H NMR spectrum. The H-15A and H-15B vinyl resonances are displaced and appear as a pair of doublets (pyridine-d_5) at δ 3.54 and 3.44 (J = 6.3 Hz) thus confirming the 4,15-epoxide. Acetylation of epoxyrepdiolide gave a diacetate **4e** which showed the oxirane doublets ($CDCl_3$) at δ 3.33 and 2.96 (J = 5 Hz). Subluteolide acetate (**4b**) [14] showed a pair of doublets ($CDCl_3$) at δ3.37 and 3.08 (J = 4.5 Hz).

To confirm the identity of 4,15-epoxyrepdiolide and establish the relative stereochemistry of the oxirane ring, epoxyrepdiolide diacetate (**4c**) was treated with *p*-toluene sulphonic acid in warm benzene to give as the major product **6**. The ready formation of the rearrangement product thus confirms the relative orientation of the oxirane ring, i.e. a 4α,15-epoxide[14]. In addition, monoepoxidation of repdiolide diacetate (**3e**) with *m*-chloroperbenzoic acid at 0° in chloroform



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gave, as the major product, 4α,15-epoxyrepdiolide diacetate identical with **4e**. Formation of **4e** is expected since the α-face of repdiolide diacetate is the less hindered side.

The relatively large amount (0.04%) of cynaropicrin (**3a**), a known cytotoxin with an ID_{50} of 5μg/ml against HeLa cells, indicates that it may well be the causative agent in ENE disease. Tests are being conducted to confirm this hypothesis and cytotoxin studies are being conducted on repdiolide and epoxyrepdiolide. The phytotoxicity of the sesquiterpenes from Russian knapweed is also being investigated.

EXPERIMENTAL

Extraction and isolation. Plant material was collected in June of 1979 near Discovery Bay along California State Highway 4 and air-dried. The ground plant (3.54 kg) was extracted sequentially with Skellysolve-F and with Et_2O to give 34 g of Et_2O extract which was dissolved in 1 l. 95% EtOH and 4% aq. Pb $(OAc)_2$ (1 l.) added. After stirring for 1 hr, the mix was filtered and the EtOH removed. Extraction with $CHCl_3$ afforded a dark coloured oil from which the sesquiterpene lactones were isolated. A portion of the oil was distilled in a sublimer at 150°/1μ pres. Aguerin B (**3c**) was isolated from the distillate by chromatography on Si gel (C_6H_6–Me_2CO) followed by prep. TLC (C_6H_6–EtOAc) and finally by prep. TLC ($CHCl_3$–MeOH). Repin (**4b**), janerin (**4c**), cynaropicrin (**3b**), repdiolide (**3a**), and epoxyrepdiolide (**4a**) were separated from the residue by a combination of CC (Si gel and LH20) and prep. TLC (Si gel) using a variety of solvent systems. The R_f values for compounds **3a–3c**, **4a–4c** are given in Table 1 with three different solvent systems. Spots were visualized by spraying with 2% aq. $KMnO_4$ soln.

Aguerin B (**3c**). Colourless oil,

$[\alpha]_{23°}^{\lambda}$ =	589	578	546	436	365
	+63.9	+68.5	+78.0	+134.4	+211.5

($CHCl_3$; *c* 0.240).

IR ν_{max}^{neat} cm^{-1}: 3450, 1762, 1720, 1640. CIMS (*iso*-butane) *m/z* (rel. int.): 331 $[M+1]^+$ (2.99), 245(44.9), 227(100).

Repin (**4b**). Colourless crystals, mp 155–157° (MeOH),

$[\alpha]_{23°}^{\lambda}$ =	589	578	546	436	365
	+93.7	+98.3	+111.7	+190.8	+300.4

($CHCl_3$; *c* 0.240).

^{1}H NMR (90 MHz, pyridine-d_5): δ1.60 (3H, *s*, H-19), 2.79 (1H, *d*, *J* = 6 Hz, H-18), 3.24 (1H, *d*, *J* = 6 Hz, H-18), 3.20 (1H, *d*, *J* = 5 Hz), 3.41 (1H, *d*, *J* = 5 Hz, H-15), 4.26 (1H, *t*, *J* = 6 Hz, H-3), 4.93 (1H, *dd*, *J* = 9, 10 Hz, H-6), 5.01 (1H, *s*, H-14), 5.17 (1H, *s*, H-14), 5.32 (1H, *m*, H-8), 5.59 (1H, *d*, *J* = 3 Hz, H-13A), 6.19 (1H, *d*, *J* = 3 Hz, H-13B). CIMS (*iso*-butane) *m/z* (rel. int.): 363 $[M+1]^+$ (87.8), 347 (11.8), 345 (11.8), 261 (100), 243 (82.7).

Janerin (**4c**). Colourless viscous oil,

$[\alpha]_{27°}^{\lambda}$ =	589	578	546	436	365
	+69.5	+72.7	+82.6	+142	+225

($CHCl_3$; *c* 2.14).

CIMS (*iso*-butane) *m/z* (rel. int.): 363 $[M+1]^+$ (33.3), 347 (4.5), 345 (7.1), 261 (100), 245 (12.7), 243 (43.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1760, 1710, 1640.

Janerin diacetate (**4d**), viscous oil,

$[\alpha]_{27°}^{\lambda}$ =	589	578	546	436	365
	+80.6	+84.2	+95.5	+162.3	+253.3

($CHCl_3$; *c* 0.640).

^{1}H NMR (90 MHz, $CDCl_3$): δ 2.04 (3H, *s*, OAc), 2.08 (3H, *s*, OAc), 2.40 (1H, *dd*, *J* = 4, 14 Hz, H-9A), 2.70 (1H, *dd*, *J* = 6, 14 Hz, H-9B), 3.04 (1H, *d*, *J* = 4 Hz, H-15), 3.29 (1H, *d*, *J* = 4 Hz, H-15), 3.43 (1H, *m*, H-7), 4.41 (1H, *dd*, *J* = 9, 11 Hz, H-6), 4.81 (2H, *s*, *H*-19), 4.93 (1H, *t*, *J* = 6 Hz, H-3), 4.96 (1H, *s*, H-14), 5.18 (1H, *s*, H-14), 5.18 (1H, *m*, H-8), 5.59 (1H, *d*, *J* = 3 Hz, H-13A), 5.94 (1H, *s*, H-18), 6.21 (1H, *d*, *J* = 3 Hz, H-13B), 6.42 (1H, *s*, H-18).

4α,15-Epoxycynaropicrin diacetate (**4d**). Cynaropicrin diacetate (**3d**), (164 mg, 0.381 mmol) was dissolved in 5 ml cold $CHCl_3$ and 82.2 mg *m*-chloroperbenzoic acid (0.381 mmol 80% acid) in 5 ml cold $CHCl_3$ was added. The soln was stirred at 0° for 18 hr then evaporated. Prep. TLC on Si gel with EtOAc–Skelly-F (1:1) followed by prep. TLC on Si gel with $CHCl_3$–MeOH (9:1) gave pure **4d** identical (IR, NMR, TLC, $[\alpha]^{\lambda}$) with acetylated janerin.

Cynaropicrin (**3b**). Colourless viscous oil,

$[\alpha]_{26°}^{\lambda}$ =	589	578	546	436	365
	+90.6	+94.6	+107.7	+183.1	+282

($CHCl_3$; *c* 1.09).

IR ν_{max}^{neat} cm^{-1}: 3400, 2940, 1759, 1640. ^{1}H NMR (90 MHz, $CDCl_3$): δ2.33 (1H, *dd*, *J* = 13.8, 3.9 Hz, H-9A), 2.70 (1H, *dd*, *J* = 13.8, 5.3 Hz, H-9B), 3.17 (1H, *ddt*, *J* = 9.6, 9.3, 3.1 Hz, H-7), 4.14 (1H, *dd*, *J* = 9.0, 9.6 Hz, H-6), 2.90 (2H, *br*, H-19), 4.47 (1H, *ddt*, *J* = 11, 7.2, 1.8 Hz, H-3), 4.90 (1H, *d*, *J* = 2.1 Hz, H-14), 5.67 (1H, *m*, H-8), 5.70 (1H, *d*, 2.1 Hz, H-14), 5.33 (1H, *br*, H-15), 5.43 (1H, *br*, H-15), 5.60 (1H, *d*, *J* = 3 Hz, H-13B), 5.93 (1H, *br*, H-18), 6.15 (1H, *d*, *J* = 3 Hz, H-13A), 6.30 (1H, *br*, H-18). ^{1}H NMR (90 MHz, DMSO-d_6): identical to the spectrum reported by Samek *et al.* [11].

Repdiolide (**3a**). Colourless viscous oil,

$[\alpha]_{25°}^{\lambda}$ =	589	578	546	436	365
	+88.4	+92.6	+106	+186	+273

($CHCl_3$; *c* 1.43).

MS (high resolution), found: 346.1422; calc. $C_{19}H_{22}O_6$, 346.1416. CIMS (*iso*-butane) *m/z* (rel. int.): 347 $[M+1]^+$ (63), 329(11), 261(33), 243(60), 233(14), 225(18), 215(46), 197(20), 147(100). IR ν_{max}^{neat} cm^{-1}: 3400, 1760, 1720, 1660, 1640. ^{13}C NMR (25.03 MHz, $CDCl_3$): δ 35.75(*t*), 46.51(*d*), 47.92(*d*), 51.36(*d*), 73.76(*d*), 77.27(*d*), 78.76(*d*), 78.88(*d*), 135.91(*t*), 137.22(*t*), 139.39(*t*), 147.22(*t*), 166.5(*s*), 169.1(*s*).

Repdiolide monoacetate. To 52.6 mg (0.152 mmol) repdiolide was added 3 ml pyridine and 16 mg (0.157 mmol) Ac_2O. The soln was stirred at room temp. for 2 hr then evaporated *in vacuo*. The resulting yellow oil was chromatographed on Si gel TLC (EtOAc) and the band at R_f 0.55 collected giving a colourless glass,

$[\alpha]_{25°}^{\lambda}$ =	589	578	546	436	365
	+71.1	+74.3	+84.1	+140	+318

($CHCl_3$; *c* 0.315).

CIMS (*iso*-butane) *m/z* (rel. int.): 389 $[M+1]^+$ (52), 371(44), 329(32), 303(70), 243(100). IR ν_{max}^{neat} cm^{-1}: 3450, 2940, 1780, 1760, 1720, 1660, 1635. ^1H NMR (90 MHz, $CDCl_3$): δ1.96 (3H, *t*, $J = 2.5$ Hz, H-19), 2.03 (3H, *s*, OAc), 2.39 (1H, *dd*, $J = 3.3$, 14.7 Hz, H-9A), 2.70 (1H, *dd*, $J = 5.4$, 14.7 Hz, H-9B), 3.17 (1H, *ddt*, $J = 9.6$, 9.6, 3.3 Hz, H-7), 4.12 (1H, *dd*, $J = 7.8$, 9.3 Hz, H-6), 4.40 (1H, *dt*, $J = 7.2$, 2.7 Hz), 4.94 (1H, *br*, H-14), 5.09 (1H, *br*, H-14), 5.60 (1H, *d*, $J = 4$ Hz, H-13A), 5.60 (1H, *br*, H-18B), 6.16 (1H, *br*, H-18A), 6.22 (1H, *d*, $J = 4$ Hz, H-13B).

Epoxyrepdiolide (**4a**). Colourless crystals, mp 129–136°,

$[\alpha]_{20°}^{\lambda}$ =	589	578	546	436	365
	+94.9	+100	+116	+199	+314

($CHCl_3$; *c* 0.215).

CIMS (*iso*-butane) *m/z* (rel. int.): 363 $[M+1]^+$ (57), 347(9), 345(15), 277(20), 259(32). IR ν_{max}^{nujol} cm^{-1}: 3500, 3300, 1765, 1715, 1655, 1635. Diacetate **4e** viscous oil,

$[\alpha]_{24°}^{\lambda}$ =	589	578	546	436	365
	+50.2	+52.0	+59.1	+101	+156

($CHCl_3$; *c*0.225).

CIMS (*iso*-butane) *m/z* (rel. int.): 447 $[M+1]^+$ (83), 387(63), 371(65), 345(22), 301(37), 259(21), 241(58), 87(100).

Epoxidation of repdiolide diacetate (**3e**). To 118 mg (0.27 mmol) repdiolide diacetate in 5 ml cold $CHCl_3$ was added 59.1 mg (0.27 mmol) 80% *m*-chloroperbenzoic acid dissolved in 5 ml cold $CHCl_3$. The resulting soln was stirred for 24 hr at 0° and then evaporated *in vacuo*. Prep. TLC on Si gel (EtOAc and Skelly solve-F-EtOAc) gave material identical (IR, NMR, TLC, HPLC) with epoxyrepdiolide diacetate (**4e**).

Acid rearrangement of epoxyrepdiolide diacetate (**4e**). To 100 mg epoxyrepdiolide diacetate (**4e**) in 5 ml C_6H_6 was added a trace (1 mg) of *p*-toluenesulphonic acid and the mixture warmed on a steam bath for 5 min. The resulting soln was evaporated over a stream of N_2 and the oil chromatographed by Si gel TLC ($CHCl_3$–MeOH, 9:1) to give 38 mg ($R_f = 0.5$) as a viscous oil.

$[\alpha]_{25°}^{\lambda}$ =	589	578	546	436	365
	+47.3	+49.2	+55.8	+92.9	+142

($CHCl_3$; *c*0.520).

CIMS (*iso*-butane) *m/z* (rel. int.): 465 $[M+1]^+$ (79), 447(68), 429(13), 405(12), 319(23), 301(23), 259(19), 241(51), 87(100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3700, 3500, 3040, 2960, 1767, 1740, 1720, 1662, 1639.

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