Tirucalla-5,24-dien-3β-ol [(13α,14β,17α,20*S*)-lanosta-5,24-dien-3β-ol]† and three other Δ^5-unsaturated tirucallanes from the roots of *Bryonia dioica* Jacq.: the first naturally occurring C-10 methylated tetracyclic triterpene alcohols with a Δ^5-monounsaturated skeleton

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Four novel triterpene alcohols with a Δ^5-unsaturated tirucallane-type skeleton, *i.e.* tirucall-5-en-3β-ol, tirucalla-5,24-dien-3β-ol, 24-methyltirucalla-5,24(24[1])-dien-3β-ol and (24*S*)-24-methyltirucalla-5,25-dien-3β-ol, have been isolated from the roots of *Bryonia dioica* Jacq. (Cucurbitaceae). The structures have been determined by spectroscopic and chromatographic methods. These compounds are the first examples of naturally occurring C-10 methylated triterpenes with a Δ^5-monounsaturated skeleton.

Introduction

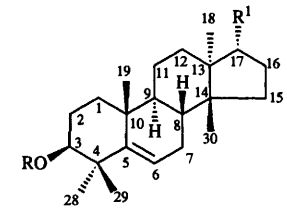
Bryonia dioica Jacq. (white bryony; Cucurbitaceae) is a climbing perennial herb with tuberous roots native to temperate Europe, North Africa and western Asia.[1] The roots of *B. dioica* are characterised by the presence of cucurbitacins, oxygenated tetracyclic triterpenes possessing a wide range of biological activities.[2] 3β-Hydroxy-D:C-*friedo*-olean-8-en-29-oic acid (3β-hydroxymultiflor-8-en-29-oic acid; bryonolic acid),[3] which has been shown to possess a marked anti-allergic activity,[4] related multiflorane-type triterpenes[5] and (24*E*)-5α-stigmasta-7,24(24[1])-dien-3β-ol (isoavenasterol)[6] have been isolated from the roots. In our continuing work on the triterpene constituents of Cucurbitaceae,[7] we now report the isolation and structure elucidation of four novel Δ^5-unsaturated tirucallane-type triterpene alcohols from the roots of *B. dioica*.

Results

The minor and trace components of the triterpene alcohol fraction of *B. dioica* included four novel compounds, *viz.* tirucall-5-en-3β-ol (**1a**; 0.1%), tirucalla-5,24-dien-3β-ol (**1b**; 1.0%), 24-methyltirucalla-5,24(24[1])-dien-3β-ol (**1c**; 0.2%) and (24*S*)-24-methyltirucalla-5,25-dien-3β-ol (**1d**; 0.2%), and a structurally related known compound, *viz.* 5α-tirucalla-7,24-dien-3β-ol (**3b**; Δ^7-tirucallol; 1.9%), a double-bond isomer of **1b**. They were isolated as the acetates (**2a**, **2b**, **2c** and **2d**) from the saponified extract of *B. dioica* roots.

Compound **2b** [*m/z* 468 (M^+, $C_{32}H_{52}O_2$)] had a secondary acetoxy group [δ_C 81.1 (d); δ_H 2.05 (3 H, s, OAc) and 4.47 (1 H, dd, *J* 7.7 and 8.1 Hz)], two trisubstituted double bonds [δ_C 121.4 (d) and 125.2 (d); δ_H 5.54 (1 H, m) and 5.10 (1 H, br t, *J* 7.0 Hz)], a terminal isopropylidene group [δ_H 1.60 (s) and 1.68 (s)], and five tertiary [δ_H 0.90 (6 H), 0.94, and 1.01 (6 H) (each s)] and one secondary (δ_H 0.88, d, *J* 6.2 Hz) methyl groups. This, in combination with fragment ions having *m/z* 453 (M^+ − Me), 397 (M^+ − Me − HOAc), 297 [loss of side-chain (C_8H_{15}) and HOAc], 241 (297 − 42 − CH_2)[8] and 69 [$CH_2CH{=}C(Me)_2]^+$, suggested that compound **2b** was a triterpene with a tetracyclic skeleton possessing one double bond, an equatorially oriented acetoxy group located most likely at C-3, and a C_8-side-chain containing an isopropylidene functionality. The highly deshielded sp^2 methine ^1H multiplet (δ 5.54) due to a skeletal double bond suggested that it was located at C-5(6).[9–13] Further, the highly deshielded ^{13}C signal at δ_C 149.1 due to C-5 quaternary carbon can be explained by the presence of seven β-substituted carbons [(6 × β^α) + (1 × β^κ)(sp^3)],[14] and was consistent with the corresponding signal in the spectrum of 4,4-dimethylcholesteryl acetate **5f** [δ_C 149.0 (C-5)]. This ruled out the possibility either of a 19(10 ⟶ 9)*abeo*-8β,9β,10α-lanost-5-ene- (10α-cucurbit-5-ene-) [δ_C 141 (C-5)],[9c] 19(10 ⟶ 9)*abeo*-8a,9β,10α-euph-5-ene- [δ_C 142 (C-5)][10] or 19(10 ⟶ 9)*abeo*-8α,9β,10α-tirucall-5-ene-[11] skeletal structure. The combined data showed that compound **2b** had a 4,4,14-trimethyl-$\Delta^{5,24}$-cholestadien-3β-yl acetate structure with an as-yet-to-be-determined stereochemistry. A weak but diagnostic mass fragment was observed at *m/z* 286 ($C_{21}H_{34}^+$), most likely involving the loss of ring A and part of ring B by cleavages of the C-1–C-10, C-5–C-10 and C-7–C-8 bonds with concomitant ^1H loss (we will refer to this fragment ion as **A**) and 271 (**A** − Me) supported the Δ^5-unsaturation of compound **2b**. Fragment **A** was observed in the mass spectra of all Δ^5-unsaturated tirucallanes and 4,4-dimethylcholesteryl acetate **5f** described in this paper. A similar ion (but formed without ^1H transfer) is a diagnostic fragment in the mass spectra of Δ^5-unsaturated sterols.[15] No fragmentation ion due to a retro-Diels–Alder cleavage of ring B, typical of triterpenoids and steroids with Δ^5-unsaturation,[16] was observed in the mass spectra of compound **2b**, other Δ^5-unsaturated tirucallanes, and **5f** described in this paper (see Experimental section). The stereochemistry of compound **2b** was determined by analysis of its 2D NMR data (^1H–^1H and ^1H–^{13}C) and by a nuclear Overhauser enhancement (NOE) study which involved a

† We chose to use the trivial names of most compounds because consistent use of systematic names would have confused the reader. Tirucallane = (13α,14β,17α,20*S*)-lanostane; euphane = (13α,14β,17α)-lanostane and cycloartane = 9,19-cyclo-9β-lanostane.



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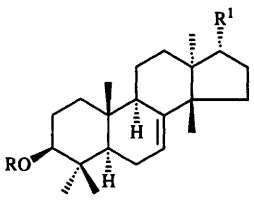

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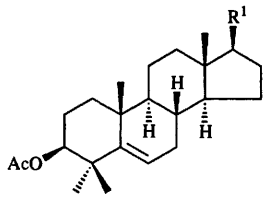

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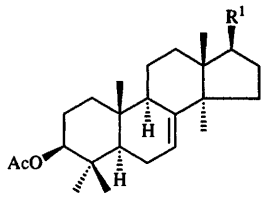

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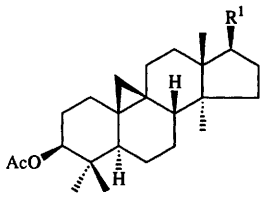

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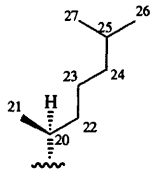
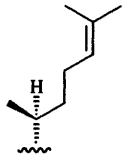
R¹ (side chain)



a (20*S*)



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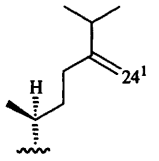
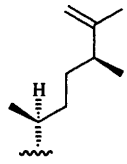
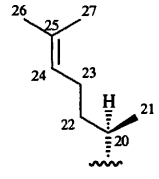


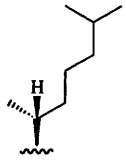
c (20*S*)



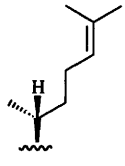
d (20*S*,24*S*)



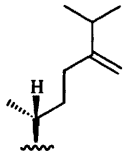
e (20*R*)



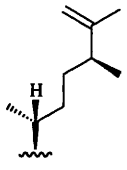
f (20*R*)



g (20*R*)



h (20*R*)



i (20*R*,24*S*)

comparison of the NOE difference effects in spectra of compound **2b** and in those of four compounds, *viz.* Δ^7-tirucallyl acetate **4b**, butyrospermyl acetate **4e**, 4,4-dimethylcholest-5-en-3β-yl (4,4-dimethylcholesteryl) acetate **5f** and 5α-lanost-7-en-3β-yl acetate **6f**. NOE Correlations are shown in Fig. 1.

Compound **5f** showed significant NOE correlation between [28-H_3(4α-Me) ~ 3α-H ~ 1α-H ~ 9α-H] on the α-face and [29-H_3(4β-Me) ~ 19-H_3(10β-Me) ~ 8β-H] on the β-face of the molecule. The same significant NOE correlation was observed also for compound **2b** demonstrating that it possessed the same stereochemistry as compound **5f** as far as rings A and B and the junction with ring C was concerned. The stereochemistry of the side-chain and of rings C and D was determined by comparison of the NOE effects in compound **2b**, the tirucallane **4b**, the euphane **4e** and the lanostane **6f**.

Compound **2b** showed NOE correlations between [19-H_3 ~ 8β-H ~ 30-H_3(14β-Me) ~ 17β-H ~ 21-H_3] on the β-face, [9α-H ~ 18-H_3(13α-Me) ~ 20-H] on the α-face, and [12α-H ~ 21-H_3]. These NOE correlations were observed also for compound **4b** although this exhibited a direct correlation between (19-H_3 ~ 30-H_3) on the β-face. The euphane **4e** showed NOE correlations between [19-H_3 ~ 30-H_3(14β-Me) ~ 17β-H ~ 21-H_3] on the β-face, [9α-H ~ 18-H_3] on the α-face, and [16α,β-H ~ 21-H_3] (Fig. 1). The lanostane **6f** showed NOE correlations between [19-H_3(10β-Me) ~ 18-H_3(13β-Me) ~ 20-H] on the β-face and between [9α-H ~ 30-H_3(14α-Me) ~ 17α-H ~ 21-H_3] on the α-face. We concluded that the structure **2b** is that of tirucalla-5,24-dien-3β-yl acetate.‡

Assigned ^{13}C and ^1H NMR data of compounds **1b**, **2b**, **4b** and **4e** are given in Tables 1 and 2, respectively.

Three other novel triterpenes were isolated as the acetates from *B. dioica* roots: compounds **2a** (*m/z* 470, M^+, $C_{32}H_{54}O_2$), **2c** (*m/z* 482, M^+, $C_{33}H_{54}O_2$) and **2d** (*m/z* 482, M^+, $C_{33}H_{54}O_2$). The ^1H NMR spectra of these acetates and the corresponding free alcohols included signals of the ring system similar to those of compounds **2b**/**1b** (see Tables 2 and 3). This suggested a tirucall- or an euph-5-en-3β-ol structure.§ The structures of the side-chains were determined by comparison of the ^1H NMR data with those of related compounds in the literature.[19]

The HPLC and GLC retention factors (R_f) of four triterpenes **2a**, **2b**, **2c** and **2d**, calculated from their relative retention times (Rt_R), were in excellent agreement with those of a set of cycloartanes, which have the same side-chains but the opposite configuration at C-20, *viz.* 5α-cycloartan-3β-yl acetate **7f**, 5α-cycloart-24-en-3β-yl acetate **7g**, 24-methyl-5α-cycloart-24(24¹)-en-3β-yl acetate **7h** and (24*S*)-24-methyl-5α-cycloart-25-en-3β-yl acetate **7i**, respectively (see Table 4). This showed that all four novel compounds were tirucallanes.

Discussion

Several naturally occurring tetracyclic[9-11] and pentacyclic triterpenes[12,13,20] with a C-9 methylated [19(10 ⟶ 9)*abeo*] Δ^5-unsaturated skeleton have been reported. However, the four tirucallane-type triterpenes **1a–d** are the first examples of triterpene alcohols with a C-10 methylated Δ^5-mono-unsaturated skeleton.¶ C-10 Methylated triterpenes with a

‡ The most stable conformation of **2b** (66.44 kcal mol^{-1}; 1 cal = 4.184 J) with minimum steric energy was simulated by using CAChe and MM2 programs[17] and is shown in Fig. 1. The same simulation was also carried out for compounds **4b** (61.03 kcal mol^{-1}), **4e** (60.72 kcal mol^{-1}) and **5f** (55.19 kcal mol^{-1}), and the results are also shown in Fig. 1. The simulated most stable conformer of compound **2b** orients C-22 in a 'right-handed' conformation (C-22 *trans*-oriented with respect to C-13) similar to that of compound **4b** and to the crystal structure of another tirucallane, 5α-tirucalla-8,24-dien-3β-yl (tirucallyl) acetate.[18] This conformation of compound **2b** was fairly consistent with results from the NOE experiment carried out in solution. In the simulated most stable conformation of compound **4e**, C-22 was *cis*-oriented ('left-handed') with respect to C-13, which was consistent with the NOE experimental results and with the crystal structure of 5α-eupha-8,24-dien-3β-yl (euphyl) acetate.[18]

§ ^1H and ^{13}C NMR data are almost useless for distinguishing between euphane- and tirucallane-type triterpenes. Examples are the NMR data of **4b**, a tirucallane, and **4e**, a euphane, which are very similar (Tables 1 and 2).

¶ The occurrence of two triterpenes with a C-10 methylated Δ^5-mono-unsaturated skeleton, *viz.* lupa-5,20(29)-dien-3β-ol in the bark of *Holarrhena antidysenterica*[21] and lupa-5,20(29)-en-3-one in the stem bark of *Pleurostylia opposita* has been reported.[22] The structural assignments of these triterpenes should be reinvestigated because they were reported to give a C-6 vinyl proton resonance at somewhat higher field, *viz.* δ 5.08[21] and 5.40,[22] respectively, in the ^1H NMR ($CDCl_3$)

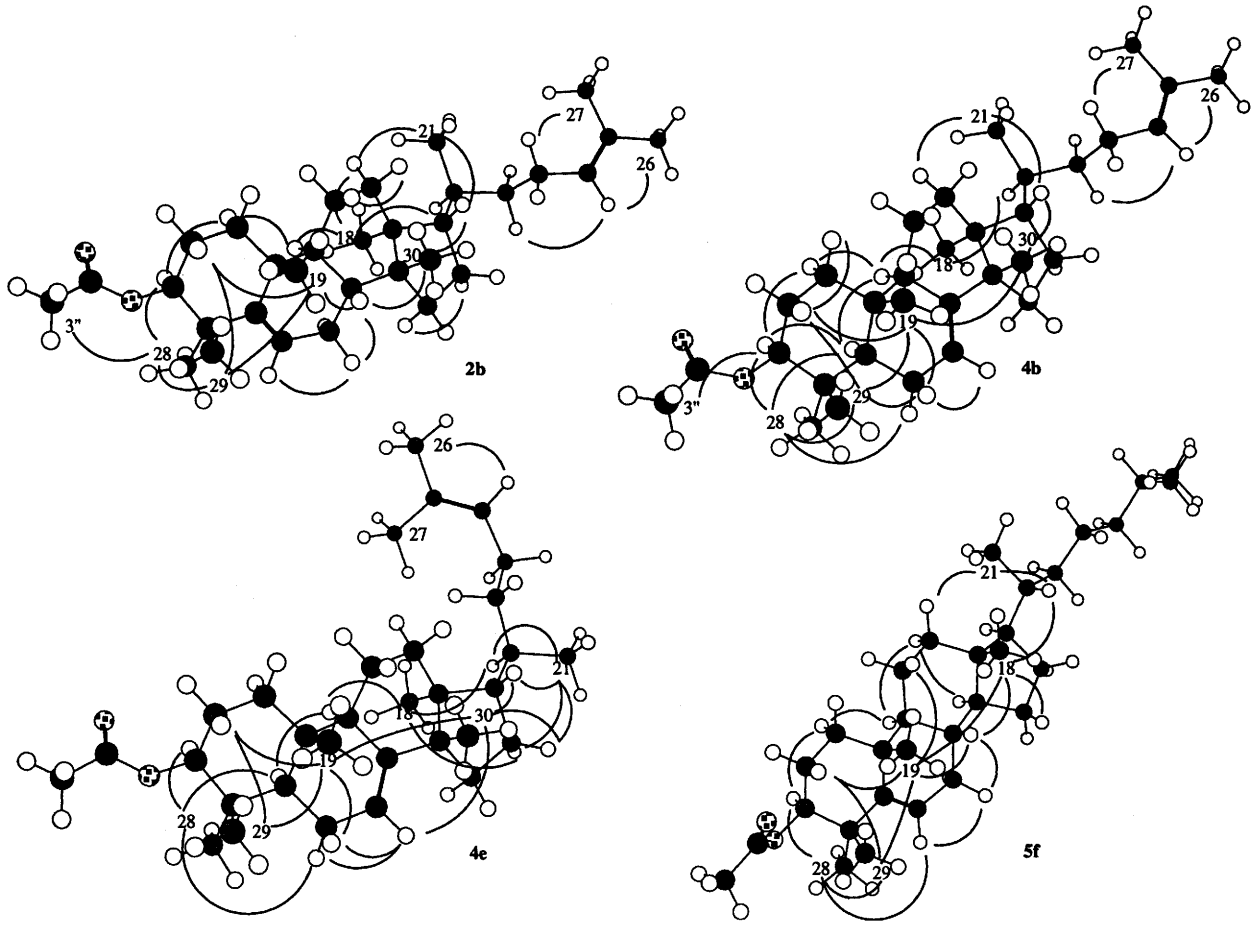


Fig. 1 CAChe drawings and some representative NOE correlations (—) for compounds **2b**, **4b**, **4e** and **5f**

Table 1 ^{13}C NMR spectral data (δ values; 100.62 MHz; $CDCl_3$) of some tirucallane- and euphane-type triterpenes with a Δ^{24}-unsaturated side-chain isolated from *B. dioica* roots

Carbon	1b	2b	4b	4e	Carbon	1b	2b	4b	4e
1	35.5	35.2	36.8	36.8	17	53.6	53.5	52.9	53.2
2	28.0	24.3	24.2	24.2	18	23.6	23.6	21.9	22.1
3	79.9	81.1	81.1	81.1	19	24.5	24.5	13.2	13.1
4	38.8	37.7	37.8	37.8	20	36.1	36.0	35.9	35.8
5	149.0	149.1	50.8	50.8	21	18.3	18.3	18.3	18.6
6	121.4	121.1	23.8	23.8	22	36.2	36.2	36.2	35.2
7	23.1	22.9	117.6	117.6	23	25.1	25.1	25.0	25.4
8	48.6	48.6	146.0	146.0	24	125.2	125.2	125.2	125.1
9	48.4	48.4	48.9	48.8	25	131.0	131.0	130.9	131.0
10	35.9	35.7	34.8	34.8	26	25.7	25.2	25.7	25.7
11	23.0	22.9	18.1	18.1	27	17.7	17.7	17.7	17.7
12	35.3	35.2	33.7	33.7	28	28.9	28.8	27.6	27.6
13	43.5	43.5	43.5	43.5	29	16.4	17.5	15.9	15.9
14	52.8	52.8	51.2	51.3	30	30.5	30.5	27.3	27.3
15	33.5	33.4	34.0	33.9	*C*OMe (3′)		171.0	171.0	171.0
16	28.7	28.6	28.2	28.5	CO*Me* (3″)		21.3	21.3	21.3

Δ^5-bond and an additional double bond in the skeleton are known constituents of the seeds of two members of the Cucurbitaceae. D:C-*friedo*-Oleana-5,7,9(11)-triene-3α,29-diol (5-dehydrokarounidiol) and 11-oxolanosta-5,16,20,25-tetraen-3β-ol (citrullonol) occur in the seeds of *Trichosanthes kirilowii*[7c] and of *Citrullus colocynthis*,[23] respectively.

It is tempting to speculate that compound **1b**, the logical precursor of compounds **1a**, **1c** and **1d**,[24] is formed by cyclisation of squalene 2,3-oxide (to give the protoeuphoid cation **8**) followed by a series of 1,2-shifts and loss of one hydrogen (see Scheme 1).‖ An alternative route might involve a C-9 carbocation and a 1,3-transannular hydrogen shift from C-5.

Experimental

General

Crystallisations were performed from methanol. Mps were measured on a Yanagimoto micro mp apparatus and are

spectrum. Δ^5-Unsaturated 3β-hydroxy (and acetoxy) triterpenes afford the C-6 vinyl proton at δ ~5.5–5.6 as shown in the literature[9–13] and in the present study. Moreover, the C-6 vinyl proton of 4,4-dimethylcholest-5-en-3-one was observed at δ 5.55 (dd, *J* 2.2 and 5.1 Hz) in the ^1H NMR ($CDCl_3$; 400 MHz) spectrum (unpublished results).

‖ It has been suggested that a series of 1,2-shifts in the protoeuphoid cation can also result in formation of the 19(10→9)*abeo*-euph-5-ene skeleton.[10]

Table 2 ^{1}H NMR Spectral data (δ values; 400 MHz; $CDCl_3$) of some tirucallane- and euphane-type triterpenes with a Δ^{24}-unsaturated side-chain isolated from *B. dioica* roots[a]

Proton	**1b**	**2b**	**4d**	**4e**
1-H_2	1.42 (2 H)	1.43(α), 1.58(β)	1.26(α), 1.66(β)	1.22(α), 1.66(β)
2-H_2	1.64 (2 H)	1.50(α), 1.66(β)	1.67 (2 H)	1.67 (2 H)
3α-H	3.21 (dd, 7.6, 7.6)	4.47 (dd, 7.7, 8.1)	4.52 (dd, 4.8, 11.4)	4.52 (dd, 4.0, 11.0)
5α-H			1.41	1.41 (dd, 5.9, 12.1)
6-H	5.56 (ddd, 2.8, 2.8, 3.6)	5.54	2.13(α), 1.93(β)	2.13(α), 1.96(β)
7-H	1.92 (2 H)	1.92 (2 H)	5.25 (dd, 2.9, 7.0)	5.25 (dd, 2.7, 6.6)
8β-H	0.85	0.92		
9α-H	2.27 (br d, 14.8)	2.28 (br d, 15.0)	2.23	2.22
11-H_2	1.70(α), 1.43(β)	1.70(α), 1.44(β)	1.52 (2 H)	1.52 (2 H)
12-H_2	1.66(α), 1.81(β)	1.63(α), 1.80(β)	1.64(α), 1.78(β)	1.66(α), 1.80(β)
15-H_2	1.50 (2 H)	1.51 (2 H)	1.53 (2 H)	1.45 (2 H)
16-H_2	1.28(α), 1.96(β)	1.26(α), 1.94(β)	1.30(α), 1.96(β)	1.27(α), 1.92(β)
17β-H	1.46	1.48	1.48	1.49
18-H_3	0.90 (s)	0.90 (s)	0.81 (s)	0.80 (s)
19-H_3	0.98 (s)	1.01 (s)	0.77 (s)	0.77 (s)
20-H	1.38	1.39	1.42	1.40
21-H_3	0.88 (d, 5.6)	0.88 (d, 6.2)	0.88 (d, 6.2)	0.85 (d, 6.2)
22-H_2	1.04, 1.44	1.04, 1.45	1.03, 1.43	0.99, 1.59
23-H_2	1.90, 2.04	1.88, 2.02	1.87, 2.04	1.88, 2.04
24-H	5.10 (br t, 7.2)	5.10 (br t, 7.0)	5.10 (br t, 7.3)	5.10 (br t, 7.0)
26-H_3	1.68 (s)	1.68 (s)	1.69 (s)	1.69 (s)
27-H_3	1.60 (s)	1.60 (s)	1.61 (s)	1.61 (s)
28-H_3	1.02 (s)	0.90 (s)	0.85 (s)	0.85 (s)
29-H_3	0.87 (s)	0.94 (s)	0.93 (s)	0.93 (s)
30-H_3	1.02 (s)	1.01 (s)	0.97 (s)	0.97 (s)
3β-OAc		2.05 (s)	2.06 (s)	2.05 (s)

[a] *J* Values (Hz) are bracketed. *J* Values not included in the Table were not determined.

Table 3 ^{1}H NMR Spectral data (δ values; 400 MHz; $CDCl_3$) of three novel tirucallane-type triterpene alcohols and their acetates reported in this paper[a]

Proton	**1a**	**2a**	**1c**	**2c**	**1d**	**2d**
3α-H	3.21	4.47 (dd-like, 7.7, 8.1)	3.21 (dd-like, 5.9, 9.8)	4.47 (dd-like, 6.0, 8.4)	3.21	4.47 (dd-like, 7.7, 8.1)
6-H	5.57	5.54	5.56 (ddd, 3.0, 3.0, 6.9)	5.55	5.55 (ddd, 2.8, 2.8, 6.6)	5.54
18-H_3	0.90 (s)	0.90 (s)	0.90 (s)	0.90 (s)	0.89 (s)	0.89 (s)
19-H_3	0.99 (s)	0.99 (s)	0.99 (s)	1.01 (s)	0.98 (s)	1.01 (s)
21-H_3	0.86 (d, 6.4)	0.86 (d, 6.4)	0.89 (d, 6.3)	0.89 (d, 6.6)	0.85 (d, 6.3)	0.85 (d, 6.3)
25-H	n.d.	n.d.	2.24 (sept., 7.4)	2.23 (sept., 6.8)		
26-H_3	0.86 (d,[b] 6.0)	0.86 (d,[b] 6.0)	1.03 (d,[b] 6.6)	1.03 (d, 6.9)	1.64 (s)	1.64 (s)
27-H	0.87 (d,[b] 6.4)	0.87 (d,[b] 6.4)	1.03 (d,[b] 6.9)	1.03 (d, 6.9)	4.67 (2 H, br s)	4.67 (2 H, br s)
24^1-H			4.66 (d, 1.4)	4.66 (d, 1.4)	1.00 (d, 6.9)	1.00 (d, 7.2)
			4.72 (s)	4.72 (s)		
28-H_3	1.02 (s)	0.90 (s)	1.02 (s)	0.90 (s)	1.02 (s)	0.90 (s)
29-H_3	0.87 (s)	0.94 (s)	0.87 (s)	0.94 (s)	0.87 (s)	0.94 (s)
30-H_3	1.02 (s)	1.02 (s)	1.02 (s)	1.03 (s)	1.02 (s)	1.01 (s)
3β-OAc		2.05 (s)		2.05 (s)		2.05 (s)

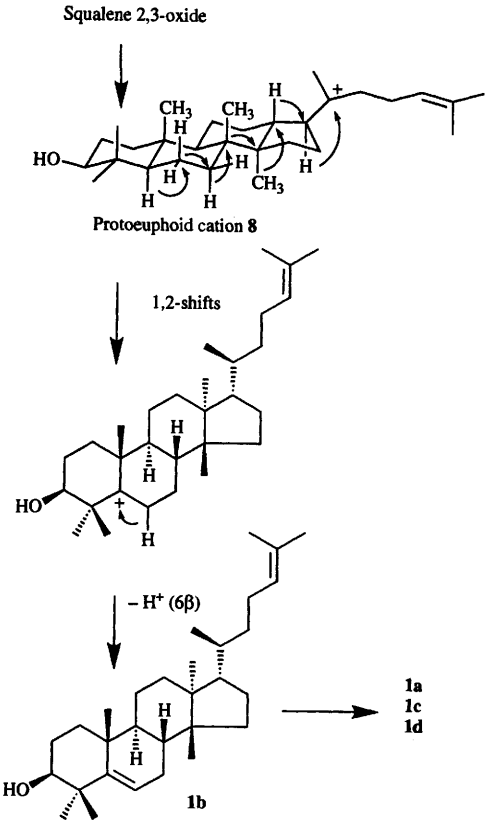
[a] *J* Values (Hz) are bracketed. *J* Values not included in the Table were not determined. [b] Assignments in each column are interchangeable. n.d. = not determined.

Table 4 Relative retention times (Rt_R)[a] and retention factor (R_f)[b] of the acetyl derivatives of some triterpene alcohols from *B. dioica* roots, and of cycloartane triterpene alcohols

	GLC		HPLC	
Triterpene acetate	Rt_R(I)	R_f	Rt_R(I)	R_f
Cycloartane group				
5α-Cycloartan-3β-ol **7f** (cycloartanol)	1.50	1.00	1.26	1.00
5α-Cycloart-24-en-3β-ol **7g** (cycloartenol)	1.82	1.21	1.01	0.80
24-Methyl-5α-cycloart-24(24^1)-en-3β-ol **7h** (24-methylenecycloartanol)	2.00	1.33	1.09	0.87
(24*S*)-24-Methyl-5α-cycloart-25-en-3β-ol **7i** (cyclolaudenol)	1.95	1.30	1.08	0.86
Δ^5-Unsaturated tirucallane group				
Tirucall-5-en-3β-ol **2a**	1.35	1.00	0.92	1.00
Tirucalla-5,24-dien-3β-ol **2b**	1.64	1.21	0.74	0.80
24-Methyltirucalla-5,24(24^1)-dien-3β-ol **2c**	1.81	1.34	0.80	0.87
(24*S*)-24-Methyltirucalla-5,25-dien-3β-ol **2d**	1.77	1.31	0.79	0.86

[a] Cholesteryl acetate has Rt_R = 1.00. [b] In each group, R_f of the triterpene acetates with a C_8-saturated side-chain (**7f** or **2a**) = 1.00.

uncorrected. Argentic TLC plates [silica gel–$AgNO_3$ (4:1)] were developed twice with CCl_4–CH_2Cl_2 (4:1). HPLC Separations were performed using an Ultrasphere ODS column (5 μ; 25 cm × 10 mm i.d., Beckman Instruments, Inc., California) using MeOH at 4 ml min^{-1} and a refractive-index detector. A DB-17 fused silica capillary column (30 m × 0.3



Scheme 1 Possible biosynthetic route for the formation of Δ^5-unsaturated tirucallane triterpene alcohols (**1a–d**)

mm i.d.; 275 °C) was used for GLC. In both HPLC and GLC, cholesteryl (cholest-5-en-3β-yl) acetate was the standard for the determination of Rt_R(I) of acetoxy triterpenes; cholesterol was the standard for the determination of Rt_R(II) for the hydroxy triterpenes. EI-MS were recorded on a Hitachi M-80B double-focussing GC–MS instrument (70 eV) using a direct inlet system. NMR Spectra were recorded with JEOL GX-400 and GSX-400 spectrometers at 400 MHz (^{1}H NMR) and 100.62 MHz (^{13}C NMR) in $CDCl_3$ with Me_4Si (^{1}H NMR) and $CDCl_3$ at δ_C 77.0 (^{13}C NMR) as internal standard. *J* Values are given in Hz. Acetylation was performed in Ac_2O–pyridine at room temperature overnight, whereas acetates were hydrolysed in 5% KOH in MeOH at room temperature overnight. The following triterpene acetates were used as reference compounds: **4b**, **4e**, **7f**, **7g**, **7h**, **7i**,[25] **5f**[26] and **6f**.[27] The roots of *B. dioica* Jacq. were collected in the Netherlands in late September, 1986.

Isolation procedure

Air-dried and ground roots of *B. dioica* (12.5 kg) were extracted with hexane and then with MeOH under reflux. Neutral lipids (5.9 g) were obtained from the combined extracts (500 g) by alkaline hydrolysis (5% KOH in MeOH; reflux; 3 h). The neutral lipids were chromatographed over silica gel (250 g) with hexane, hexane–EtOAc (9:1, v/v) and hexane–EtOAc (4:1) as eluents. The residue of the hexane–EtOAc (9:1) eluate yielded a triterpene alcohol fraction (376 mg) after rechromatography over silica gel. The fraction was acetylated, and the resulting acetate fraction (374 mg) was subjected to argentic TLC followed by HPLC. The following triterpene acetates discussed in this paper were obtained: **2a** (0.2 mg), **2b** (3.6 mg), **2c** (0.7 mg), **2d** (0.5 mg), **4b** (7.0 mg), **4e** (11 mg), **7g** (55 mg) and **7h** (18 mg). Known triterpenes (**4b**, **4e**, **7g** and **7h**) were identified by chromatographic (HPLC, GLC) and spectral (mass, ^{1}H NMR) comparison with reference compounds. All identified triterpene alcohols, 4α-methyl sterols and sterols isolated from *B. dioica* roots have been reported in another paper.[28]

Tirucall-5-en-3β-yl acetate 2a and tirucall-5-en-3β-ol 1a. *Compound* **2a**: *m/z* (assignment) 470.4111 ($C_{32}H_{54}O_2$, M^+, 6%; requires *M*, 470.4120), 455.3889 ($C_{31}H_{51}O_2$, 26), 395.3628 ($C_{29}H_{27}$, 18), 315.2382 ($C_{21}H_{31}O_2$, 1), 297.2569 ($C_{22}H_{33}$, 1), 288.2797 ($C_{21}H_{36}$, **A**, 3), 283.2438 ($C_{21}H_{31}$, 1), 273.2543 ($C_{22}H_{33}$, **A** – Me, 5), 241.1985 ($C_{18}H_{25}$, 2), 229.1991 ($C_{17}H_{25}$, 5), 43.0578 (C_3H_7) and 43.0217 (C_2H_3O, 100). Alkaline hydrolysis of compound **2a** afforded the alcohol **1a**, Rt_R(II) (GLC) 1.48; *m/z* 428.3987 ($C_{30}H_{52}O$, M^+, 2%; requires *M*, 428.4015), 413.3819 ($C_{29}H_{49}O$, 5), 395.3695 ($C_{29}H_{47}$, 3), 288.2798 ($C_{21}H_{36}$, **A**, 3), 273.2518 ($C_{20}H_{33}$, **A** – Me, 6), 259.2090 ($C_{18}H_{27}O$, 3), 255.2138 ($C_{19}H_{27}$, 5), 241.1980 ($C_{18}H_{25}$, 2), 229.2011 ($C_{17}H_{25}$, 4) and 43.0542 (C_3H_7, 100).

Tirucalla-5,24-dien-3β-yl acetate 2b and tirucalla-5,24-dien-3β-ol 1b. *Compound* **2b**: mp 138–140 °C; *m/z* 468.3938 ($C_{32}H_{52}O_2$, M^+, 24%; requires *M*, 468.3964), 453.3689 ($C_{31}H_{49}O_2$, 47), 408.3629 ($C_{30}H_{48}$, 2), 393.3477 ($C_{29}H_{45}$, 37), 315.2361 ($C_{21}H_{31}O_2$, 2), 297.2555 ($C_{22}H_{33}$, 3), 295.2407 ($C_{22}H_{31}$, 1), 286.2665 ($C_{21}H_{34}$, **A**, 3), 271.2424 ($C_{20}H_{31}$, **A** – Me, 9), 257.2244 ($C_{19}H_{29}$, 4), 255.2062 ($C_{19}H_{27}$, 5), 241.1907 ($C_{18}H_{25}$, 7) and 69.0695 (C_5H_9, 100). Alkaline hydrolysis of acetate **2b** yielded the alcohol **1b**, mp, 139–140 °C, Rt_R(II) (GLC) 1.78; *m/z* 426.3847 ($C_{30}H_{50}O$, M^+, 13%; requires *M*, 426.3859), 411.3610 ($C_{29}H_{47}O$, 25), 393.3508 ($C_{29}H_{45}$, 7), 341.2787 ($C_{24}H_{37}O$, 1), 286.2673 ($C_{21}H_{34}$, 3), 271.2413 ($C_{20}H_{31}$, **A** – Me, 4), 259.2122 ($C_{18}H_{27}O$, 2), 255.2062 ($C_{19}H_{27}$, 1), 243.2087 ($C_{18}H_{27}$, 2), 241.1924 ($C_{18}H_{25}$, 2), 229.1990 ($C_{17}H_{25}$, 2), 215.1828 ($C_{16}H_{25}$, 3), 201.1670 ($C_{15}H_{21}$, 5) and 69.0706 (C_5H_9, 100).

The NOE correlations for acetate **2b** shown in Fig. 1 were determined using the difference NOE spectral technique. The representative correlations observed were as follows. Irradiation of the signal at δ 4.47 (3α-H) enhanced signals at δ 0.90 [28-H_3 (4α-Me)] and 1.43 (1α-H). Irradiation of the signal at δ 1.01 [19-H_3 and 30-H_3 (14β-Me)] enhanced signals at δ 0.92 (8β-H), 0.94 [29-H_3 (4β-Me)] and 1.48 (17β-H), whereas irradiation at δ 0.92 (8β-H) enhanced a signal at δ 1.01 (19-H_3 and 30-H_3). Irradiation at δ 1.48 (17β-H) enhanced a signal at δ 1.01 (30-H_3). Irradiation at δ 0.88 (21-H_3) enhanced signals at δ 1.39 (20-H), 1.48 (17β-H) and 1.63 (12β-H). Irradiation at δ 2.28 (9α-H) enhanced a signal at δ 0.90 [18-H_3 (13α-Me)]. Finally, irradiation of the signal at δ 0.90 (18-H_3 and 28-H_3) enhanced signals at δ 1.39 (20-H) and 4.47 (3α-H). The presence of two overlapped methyl signals at δ 0.90 (18-H_3 and 28-H_3) and 1.01 (19-H_3 and 30-H_3) in the NMR spectrum of acetate **2b** caused some ambiguity in the assignment of NOE correlations, which was overcome by comparison of the NOE correlations observed for free alcohol **1b**. The representative NOE correlations for alcohol **1b** were as follows. Irradiation of the signal at δ 3.21 (3α-H) enhanced signals at δ 1.02 (28-H_3) and 1.42 (1α-H). Irradiation at δ 0.98 (19-H_3) enhanced signals at δ 0.85 (8β-H) and 0.87 (29-H_3), whereas irradiation at δ 0.85 (8β-H) enhanced signals at δ 0.98 (19-H_3) and 1.02 (30-H_3). Further irradiation at δ 1.02 (28-H_3 and 30-H_3) enhanced signals at δ 0.85 (8β-H), 0.87 (29-H_3), 1.46 (17β-H) and 3.21 (3α-H), while irradiation at δ 1.46 (17β-H) enhanced a signal at δ 1.02 (30-H_3). Irradiation at δ 0.88 (21-H_3) enhanced signals at δ 1.38 (20-H) and 1.66 (12β-H). Irradiation at δ 0.90 (18-H_3) enhanced signals at δ 1.38 (20-H) and 2.27 (9α-H), whereas irradiation at δ 2.27 (9α-H) enhanced signals at δ 0.90 (18-H_3) and 1.42 (1α-H).

24-Methyltirucalla-5,24(24^1)-dien-3β-yl acetate 2c and 24-methyltirucalla-5,24(24^1)-dien-3β-ol 1c. *Compound* **2c**: *m/z* 482.4123 ($C_{33}H_{54}O_2$, M^+, 9%; requires *M*, 482.4121), 467.3880 ($C_{32}H_{51}O_2$, 21), 422.3841 ($C_{31}H_{50}$, 1), 407.3651 ($C_{30}H_{47}$, 7), 383.2941 ($C_{26}H_{39}O_2$, 1), 369.2778 ($C_{25}H_{37}O_2$, 1), 355.2671 ($C_{24}H_{35}O_2$, 1), 323.2740 ($C_{24}H_{35}$, 2), 315.2273 ($C_{21}H_{31}O_2$, 1), 301.2173 ($C_{20}H_{29}O_2$, 4), 300.2792 ($C_{22}H_{36}$, **A**, 1), 297.2575 ($C_{22}H_{33}$, 2), 285.2535 ($C_{21}H_{33}$, **A** – Me, 3), 283.2382 ($C_{21}H_{31}$, 3), 255.2129 ($C_{19}H_{27}$, 3), 241.1982 ($C_{17}H_{25}$, 4) and 43 (100). Alkaline hydrolysis of acetate **2c** yielded *alcohol* **1c**, mp 165–167 °C, Rt_R(II) (GLC) 1.95; *m/z* 440.3992 ($C_{31}H_{52}O$, M^+, 11%;

requires *M*, 440.4015), 425.3734 ($C_{30}H_{49}O$, 27), 407.3651 ($C_{30}H_{47}$, 5), 393.3447 ($C_{29}H_{45}O$, 1), 341.2878 ($C_{24}H_{37}O$, 3), 323.2727 ($C_{24}H_{35}$, 2), 300.2805 ($C_{22}H_{36}$, **A**, 4), 285.2525 ($C_{21}H_{33}$, **A** − Me, 4), 273.2289 ($C_{19}H_{29}O$, 3), 259.2089 ($C_{18}H_{27}O$, 7), 255.2132 ($C_{19}H_{27}$, 3), 241.1983 ($C_{18}H_{25}$, 6), 229.2002 ($C_{17}H_{25}$, 5) and 55.0543 (C_4H_7, 100).

(24*S*)-24-Methyltirucalla-5,25-dien-3β-yl acetate 2d and (24*S*)-24-methyltirucalla-5,25-dien-3β-ol 1d. *Compound* **2d**: *m/z* 482.4106 ($C_{33}H_{54}O_2$, M^+, 17%; requires *M*, 482.4121), 467.3867 ($C_{32}H_{51}O_2$, 27), 422.3847 ($C_{31}H_{50}$, 2), 407.3647 ($C_{30}H_{47}$, 18), 397.3067 ($C_{27}H_{41}O_2$, 1), 357.2745 ($C_{24}H_{37}O_2$, 1), 337.2898 ($C_{24}H_{37}$, 3), 315.2388 ($C_{21}H_{31}O_2$, 2), 301.2171 ($C_{20}H_{29}O_2$, 4), 300.2716 ($C_{22}H_{36}$, **A**, 3), 297.2536 ($C_{22}H_{33}$, 3), 285.2598 ($C_{21}H_{33}$, **A** − Me, 5), 283.2437 ($C_{21}H_{31}$, 12), 255.2063 ($C_{19}H_{27}$, 5), 241.1992 ($C_{17}H_{25}$, 5), 229.1971 ($C_{17}H_{25}$, 7) and 43 (100). Alkaline hydrolysis of acetate **2d** yielded *alcohol* **1d**, mp 146–148 °C, Rt_R(II) (GLC) 1.94; *m/z* 440.4011 ($C_{31}H_{52}O$, M^+, 8%; requires *M*, 440.4015), 425.3786 ($C_{30}H_{49}O$, 17), 407.3630 ($C_{30}H_{47}$, 4), 355.2987 ($C_{25}H_{39}O$, 1), 341.2810 ($C_{24}H_{37}O$, 2), 311.2328 ($C_{22}H_{31}O$, 3), 300.2789 ($C_{22}H_{36}$, **A**, 4), 285.2552 ($C_{21}H_{33}$, **A** − Me, 4), 283.2390 ($C_{21}H_{31}$, 6), 273.2315 ($C_{19}H_{29}O$, 2), 271.2217 ($C_{19}H_{27}O$, 2), 259.2106 ($C_{18}H_{27}O$, 5), 255.2132 ($C_{19}H_{27}$, 2), 241.1983 ($C_{18}H_{25}$, 4), 229.2005 ($C_{17}H_{25}$) and 41.0385 (C_3H_5, 100).

4,4-Dimethylcholest-5-en-3β-yl acetate 5f. This compound showed *m/z* 456.3941 ($C_{31}H_{52}O_2$, M^+, 15%; Calc. for *M*, 456.3963), 441.3706 ($C_{30}H_{49}O_2$, 1), 396.3720 ($C_{29}H_{48}$, 23), 381.3523 ($C_{28}H_{45}$, 38), 353.3159 ($C_{26}H_{41}$, 3), 328.3101 ($C_{24}H_{40}$, 12), 313.2898 ($C_{23}H_{37}$, 2), 283.2450 ($C_{21}H_{31}$, 6), 274.2621 ($C_{20}H_{34}$, **A**, 4), 259.2421 ($C_{19}H_{31}$, **A** − Me, 1), 247.2405 ($C_{18}H_{31}$, 5), 241.1954 ($C_{18}H_{25}$, 6), 227.1844 ($C_{17}H_{23}$, 2), 215.1828 ($C_{16}H_{23}$, 4) and 43 (100); δ_C and δ_H: C-1 [36.3; 1.18(α), 1.74(β)], C-2 [23.9; 1.73(β), 1.83(α)], C-3 [79.5; 4.49, dd, *J* 4.4 and 11.7], C-4 [40.4], C-5 [149.0], C-6 [120.7; 5.56, dd, *J* 2.9 and 4.4], C-7 [32.5; 1.64(α), 2.09(β)], C-8 [30.9; 1.49], C-9 [50.8; 0.92], C-10 [36.7], C-11 [20.6; 1.36(β), 1.47(α)], C-12 [39.7; 1.15(α), 2.00(β)], C-13 [42.2], C-14 [57.2; 0.94], C-15 [23.8; 1.17(α), 1.33(β)], C-16 [28.3; 1.26(β), 1.84(α)], C-17 [56.0; 1.06], C-18 [11.9; 0.67, s], C-19 [21.4; 1.10, s], C-20 [35.8; 1.37], C-21 [18.7; 0.91, d, *J* 6.2], C-22 [36.2; 1.00, 1.34]; C-23 [24.2; 1.07, 1.59], C-24 [39.5; 1.11, 1.11], C-25 [28.0; 1.52], C-26 [22.6; 0.86, d, *J* 6.6], C-27 [22.8; 0.87, d, *J* 6.6], C-28 [27.2; 1.02, s], C-29 [25.0; 1.14, s], C-3′ (*C*OMe) [170.8] and C-3″ (CO*Me*) [21.4; 2.06, s].

5α-Lanost-7-en-3β-yl acetate 6f. δ_C and δ_H: C-1 [37.7; 1.28(α), 1.79(β)], C-2 [24.0; 1.64, 1.64], C-3 [81.2; 4.52, dd-like, *J* 4.7 and 10.7], C-4 [37.5], C-5 [50.3; 1.22], C-6 [22.8; 1.91(β), 2.02(α)], C-7 [116.4; 5.20, br d, *J* 5.5], C-8 [145.1], C-9 [47.1; 2.01], C-10 [35.5], C-11 [20.0; 1.62(β), 1.48(α)], C-12 [32.1; 1.65, 1.65], C-13 [44.3], C-14 [52.0], C-15 [32.2; 1.23(β), 1.57(α)], C-16 [27.6; 1.29(α), 1.95(β)], C-17 [50.8; 1.49], C-18 [16.0; 0.64, s], C-19 [14.2; 0.89, s], C-20 [36.5; 1.35], C-21 [19.0; 0.88, d, *J* 6.6], C-22 [36.5; 1.00, 1.15], C-23 [24.1; 1.18, 1.32], C-24 [39.5; 1.13, 1.13], C-25 [28.0; 1.52], C-26 [22.6; 0.86, d, *J* 6.6], C-27 [22.8; 0.87, d, *J* 6.6], C-28 [28.2; 0.87, s], C-29 [16.6; 0.96, s], C-30 [24.8; 0.97, d, *J* 0.7], C-3′ (*C*OMe) [171.1] and C-3″ (CO*Me*) [21.3; 2.05, s].

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