Eight Novel Sterols from the Roots of *Bryonia dioica* JACQ.

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Eight novel sterols, 4,4-dimethyl-5α-poriferasta-7,25-dien-3β-ol, (24*E*)-24-ethyl-29-nor-5α-lanosta-7,24(24[1])-dien-3β-ol, (24*E*)-24-ethyl-29-nor-5α-lanosta-9(11),24(24[1])-dien-3β-ol, (24*E*)-24-ethyl-29-nor-5α-lanosta-7,9(11),24(24[1])-trien-3β-ol, (22*E*,24*Z*)-5α-stigmasta-7,22,24(24[1])-trien-3β-ol, 5α-stigmasta-7,9(11)-dien-3β-ol, (24*E*)-5α-stigmasta-7,9(11),24(24[1])-trien-3β-ol, and (24E)-14α-methyl-5α-stigmasta-7,24(24[1])-dien-3β-ol, were isolated (as acetates) from the saponified neutral fraction of *Bryonia dioica* root extract. The structures were determined by spectroscopic methods. The compounds are new because the 4,4-dimethyl-Δ^7-cholestene-, 4α,14α-dimethyl-Δ^7-cholestene (or 29-nor-Δ^7-lanostene)-, and 14α-methyl-Δ^7-cholestene-skeletons and the (22*E*,24*Z*)-$\Delta^{22,24(24^1)}$-stigmastadiene side chain have not been found before in nature. Abundances are given of fifty three identified triterpene alcohols and sterols.

Key words *Bryonia dioica*; Cucurbitaceae; root; sterol; triterpene alcohol; NMR spectroscopy

Bryonia dioica JACQ. (white bryony; Cucurbitaceae) is a climbing perennial herb with tuberous roots which occurs in temperate Europe, North Africa, and western Asia.[1] The roots of *B. dioica* are characterized 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 (bryonolic acid)[3] and related D:C-*friedo*-oleanane-type triterpenes,[4] and 5α-stigmast-7-en-3β-ol (schottenol), (22*E*)-5α-stigmasta-7,22-dien-3β-ol (spinasterol), and (24*E*)-5α-stigmasta-7,24-(24[1])-dien-3β-ol (isoavenasterol)[5] have been isolated from the roots. Continuing our work on the sterol[6] and triterpene constituents[7] of the Cucurbitaceae, we now report the isolation and structure elucidation of eight novel sterols.

Eight novel sterols were isolated from the saponified neutral fraction of *B. dioica* root extract (see Experimental for details). The acetate of 4,4-dimethyl-5α-poriferasta-7,25-dien-3β-ol (**1**) was isolated from the (acetylated) triterpene alcohol fraction; the acetates of (24*E*)-24-ethyl-29-nor-5α-lanosta-7,24(24[1])-dien-3β-ol (**2**), (24*E*)-24-ethyl-29-nor-5α-lanosta-9(11),24(24[1])-dien-3β-ol (**3**), and (24*E*)-24-ethyl-29-norlanosta-7,9(11),24(24[1])-trien-3β-ol (**4**) were isolated from the (acetylated) 4α-methylsterol fraction, and (22*E*,24*Z*)-5α-stigmasta-7,22,24(24[1])-trien-3β-ol (**5**), 5α-stigmasta-7,9(11)-dien-3β-ol (**6**), (24*E*)-5α-stigmasta-7,9(11),24(24[1])-trien-3β-ol (**7**) and (24*E*)-14α-methyl-5α-stigmasta-7,24(24[1])-dien-3β-ol (**8**) from the (acetylated) sterol fraction, respectively. Their structures are shown in Chart 1.

All novel sterols have a secondary acetoxyl group [^{1}H: δ 2.03—2.06 (3H, s), OCOMe] associated with an adjacent methine [^{1}H: δ *ca.* 4.4—4.7 (1H, dd, dt, or tt), J=*ca.* 4—5, 10—11 Hz] (Table 1). The shift and coupling constants of the methine ^{1}H signal showed that the acetoxyl group of the eight steryl acetates is oriented equatorially (β) at C-3.

High-resolution mass spectrum (HR-MS) of **1a** (the acetate of **1**) showed M^+ at m/z 482.4126 ($C_{33}H_{54}O_2$) and prominent fragment ions at m/z 407 ($M^+ - Me - HOAc$) and 341 [M^+ − side chain (s.c.; $C_{10}H_{19}$) −2H], 301 [M^+ − s.c. − 42 (ring D; C_3H_6)],[8] 283 (M^+ − s.c. − HOAc), 281 (341 − HOAc), and 241 (301 − HOAc). This indicated that **1a** was an acetate of a C_{31} sterol containing an unsaturation, one is in the C_{10} side chain and the other in the skeleton with two additional methyl groups. Absence of fragments having m/z 287 (M^+ − s.c. − 42 − CH_2) and 227 (287 − HOAc) eliminated the possibility of the presence of a methyl group at C-14.[8] A diagnostic ion observed at m/z 257, probably caused by loss of part of ring D by cleavages of the C-13–C17 and C-15–C-16 bonds with concominant HOAc loss (M^+ − s.c. − 26 − HOAc),[8,9] suggested that **1a** possessed a 4,4-dimethyl-Δ^7-sterol skeleton. The proposed skeletal structure was supported by the ^{1}H-NMR spectral data since the ^{1}H signals arising from A- and B-ring protons (H-3α, OAc-3β, H-7, H-19, H-28, H-29) of **1a** (Table 1) were in good agreement with those of synthetic 5α-lanost-7-en-3β-yl acetate.[10] On the other hand, the ^{1}H signals of the side chain protons of **1a** were consistent with those of 5α-poriferasta-7,25-dien-3β-yl acetate.[11] These data suggested that **1a** was 4,4-dimethyl-5α-poriferasta-7,25-dien-3β-yl acetate. Alkaline hydrolysis of **1a** yielded a free sterol **1** which showed M^+ at m/z 440.4035 ($C_{31}H_{52}O$) in the HR-MS. The skeletal methyl ^{1}H signals (H-18, H-19, H-28, and H-29) of **1** in the ^{1}H-NMR spectrum (see Experimental section) were consistent with the corresponding signals reported for synthetic 4,4-dimethyl-5α-cholest-7-en-3β-ol,[12] thus confirming the structure of **1** and its acetate **1a**.

The molecular formulae of 4α-methylsteryl acetates **2a** (M^+, m/z 482.4106) and **3a** (M^+, m/z 482.4140) were both determined to be $C_{33}H_{54}O_2$ (HR-MS), whereas the molecular formula of **4a** (M^+, m/z 480.3944) was $C_{33}H_{52}O_2$. Thus all three compounds were C_{31} sterols, **2a** and **3a** having two degrees of unsaturation and **4a** having three degrees of unsaturation. The mass spectra of all three compounds showed prominent ions [M^+ − s.c. ($C_{10}H_{19}$) − 2H] (m/z 341 for **2a** and **3a**, and m/z 339 for

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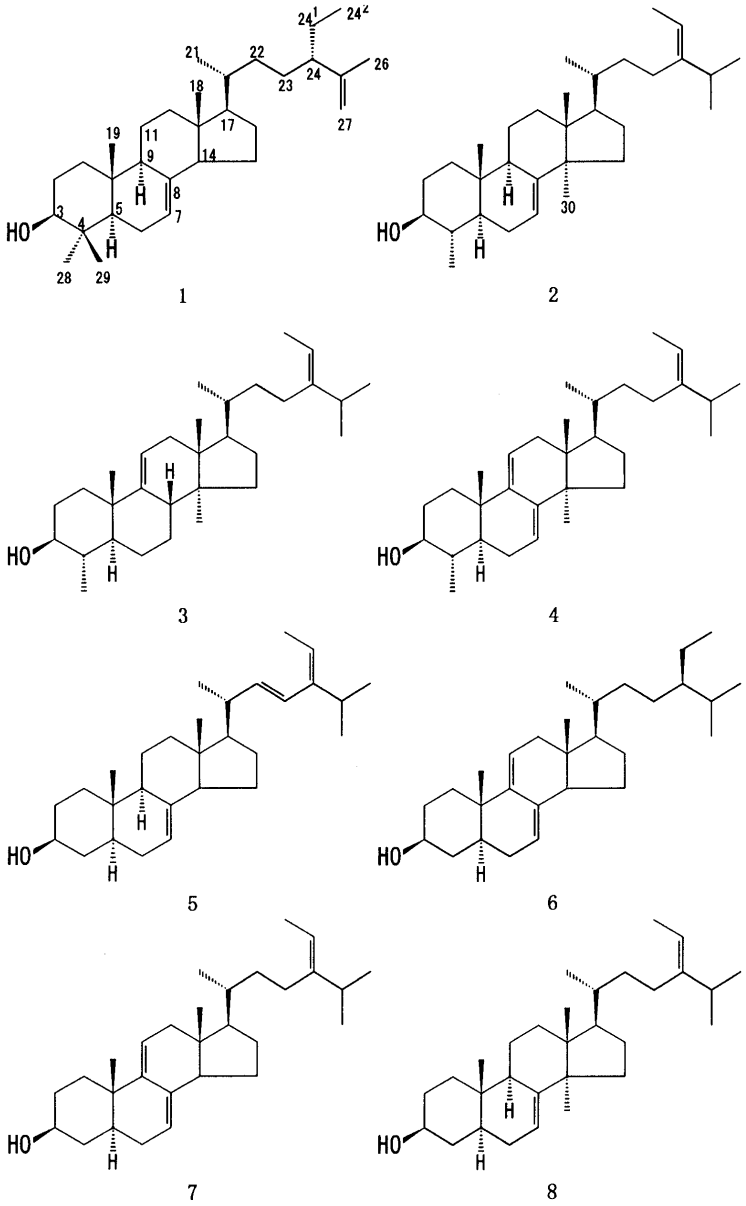

Chart 1. Structures of Eight Novel Sterols from *Bryonia dioica* Roots

4a), [$M^+ - s.c. - 42$ (ring D)], and ($M^+ - s.c. - 42 - CH_2$), indicating that all these sterols had a mono-unsaturated C_{10} side chain and a methyl group at C-14[8]; **2a** and **3a** had monounsaturated skeleton and **4a** a di-unsaturated skeleton. Occurrence of further fragments [$M^+ - C_7H_{14}$ (part of side chain)] (*m/z* 384 for **2a** and **3a**, and *m/z* 382 for **4a**) and ($M^+ - C_7H_{14} - Me$), formed by the allylic cleavage of the C-22–C-23 bond due to the McLafferty-type rearrangement,[8] limited the possibilities for the position of the double bond in the side chain to $\Delta^{24(24^1)}$ or $\Delta^{24(25)}$. All three sterols exhibited the side chain ^1H signals at 1.58 (3H, d, H-24^2), 2.21 (1H, sept, H-25), and 5.19 (1H, q, H-24^1) in the ^1H-NMR spectra (Table 1) which were in agreement with the corresponding signals for isocitrostadienyl acetate,[13] thus revealing that these have a (24*E*)-ethylidene substituted (C24^2 *cis* to C23) side chain. Comparison of the skeletal ^1H signals of **2a** and **3a** (Table 1) with those of 29-nor-5α-lanost-7-en-3β-yl acetate[10] and (24*Z*)-24-ethyl-29-nor-5α-lanosta-9(11),24-(24^1)-dien-3β-yl acetate,[13] respectively, left no doubt that **2a** and **3a** had a 29-nor-5α-lanost-7-en-3β-yl and 29-nor-5α-lanost-9(11)-en-3β-yl acetate skeletal structures, respectively. The combined MS and ^1H-NMR information confirmed that **2a** and **3a** were (24*E*)-24-ethyl-29-nor-5α-lanosta-7,24(24^1)-dien-3β-yl acetate and (24*E*)-24-ethyl-29-nor-5α-lanosta-9(11),24(24^1)-dien-3β-yl acetate, respectively. Acetate **4a** showed an UV absorption (in EtOH) at 233, 242, and 250 nm typical of the presence of a $\Delta^{7,9(11)}$-diene system in the sterol skeleton.[14] **4a** also showed almost the same skeletal ^1H signals in the ^1H-NMR spectrum (Table 1) to 24-methyl-29-nor-5α-lanosta-7,9(11),24(24^1)-trien-3β-yl acetate.[3] Thus the combined data proved that **4a** was (24*E*)-24-ethyl-29-nor-5α-lanosta-7,9(11),24(24^1)-trien-3β-yl acetate.

Acetate **5a** was an acetate of C_{29} sterol with three degrees of unsaturation [M^+, *m/z* 452.3678 ($C_{31}H_{48}O_2$)]. The MS exhibited a diagnostic fragment at *m/z* 313 ($M^+ - s.c. - 2H$) indicating that it possessed a di-unsaturated C_{10} side chain and a mono-unsaturated skeleton. Another prominent fragment in the MS had *m/z* 342 ($M^+ - C_8H_{14}$)

Table 1. ^{1}H-NMR Data (δ/ppm, 400 MHz, $CDCl_3$) of the Acetates of Eight Novel Sterols from the Saponified Neutral Fraction of *Bryonia dioica* Root Extract[a)]

Proton	Steryl acetate							
	1a	**2a**	**3a**	**4a**	**5a**[b)]	**6a**	**7a**	**8a**
3	4.51 (dd) (4.8, 10.3)	4.40 (dt) (4.4, 11.0)	4.37 (dt) (5.1, 11.0)	4.40 (dt) (4.4, 11.0)	4.70 (tt) (4.7, 11.3)	4.68 (tt) (4.6, 11.3)	4.68 (tt) (4.7, 11.3)	4.69 (tt) (4.7, 11.5)
OAc-3	2.05 (s)	2.05 (s)	2.06 (s)	2.06 (s)	2.03 (s)	2.03 (s)	2.03 (s)	2.03 (s)
7	5.20 (m) (8.9)	5.17 (br d) (5.5)	n.d.	5.41 (br d) (7.7)	5.15 (dt) (4.4, 2.0)	5.35 (br s)	5.37 (br s)	5.15 (dt) (4.8, 2.2)
11	n.d.	n.d.	5.31 (br d) (7.0)	5.39 (br d) (7.7)	n.d.	5.45 (br d) (7.4)	5.46 (br d) (6.3)	n.d.
18	0.51 (s)	0.67 (s)	0.66 (s)	0.59 (s)	0.58 (s)	0.50 (s)	0.51 (s)	0.67 (s)
19	0.88 (s)	0.86 (s)	1.00 (s)	0.96 (s)	0.82 (s)	0.92 (s)	0.92 (s)	0.83 (s)
21	0.91 (d) (6.6)	0.96 (d) (6.3)	0.95 (d) (6.2)	0.96 (d) (6.9)	1.08 (d) (6.6)	0.92 (d) (5.8)	0.99 (d) (6.6)	0.96 (d) (6.6)
25	—	2.21 (sept) (6.9)	2.21 (sept) (7.0)	2.21 (sept) (6.3)	2.55 (sept) (6.6)	n.d.	2.19 (sept) (6.9)	2.21 (sept) (6.9)
26	1.57 (dd) (0.6, 1.4)	0.98 (d) (6.1)	0.98 (d)[c)] (7.0)	0.99 (6.9)	1.02 (d)[c)] (6.6)	0.84 (d) (7.1)	0.98 (d)[c)] (6.9)	0.98 (d)[c)] (6.9)
27	4.64 (br d, 2.5) 4.73 (dt; 2.5, 1.4)	0.98 (d) (6.1)	0.99 (d)[c)] (7.0)	0.99 (d) (6.9)	1.03 (d)[c)] (6.9)	0.82 (d) (6.4)	0.99 (d)[c)] (6.9)	0.99 (d)[c)] (6.9)
24^1	n.d.	5.18 (q) (6.6)	5.19 (q) (7.0)	5.19 (q) (6.6)	5.32 (q) (6.9)	n.d.	5.19 (q) (6.6)	5.19 (q) (6.3)
24^2	0.80 (t) (7.3)	1.58 (d) (6.9)	1.58 (d) (6.6)	1.58 (d) (6.6)	1.71 (d) (7.2)	0.85 (t) (7.1)	1.58 (d) (6.9)	1.58 (d) (6.9)
28	0.99 (s)	0.85 (d) (6.6)	0.84 (d) (6.2)	0.87 (d) (6.3)	—	—	—	—
29	0.86 (s)	—	—	—	—	—	—	—
30	—	0.99 (s)	0.74 (s)	0.89 (s)	—	—	—	0.99 (s)

a) Figures in parentheses denote *J* values (Hz) with the exception of the multiplet signals where the figures denote $W_{1/2}$ values (Hz). n.d. = not determined. *b*) Other signals: H-22 (δ 5.51, dd, J = 8.8, 15.9 Hz), H-23 (δ 6.17, d, J = 15.7 Hz). *c*) Assignment interchangeable.

[with daughter ions at *m/z* 327 (342 − Me) and 282 (342 − HOAc)] formed by vinylic cleavage of the C-20–C-22 bond with ^{1}H transfer. This suggested the presence of a Δ^{22}-double bond in the C_{10} side chain.[15,16)] The other double bond was part of an ethylidene group (vinylic methyl at δ 1.71) and located at C-24(24^1) since the MS of **5a** included a diagnostic peak at *m/z* 409 ($M^+ - C_3H_7$) due to the loss of the terminal isopropyl group (C-25–C-27).[15)] The UV absorption at 240 nm (in EtOH) was indicative of a *S-cis* diene chromophore.[17a)] Using nuclear Overhauser effect (NOE) experiments (see Experimental), the double bond configuration of the ethylidene group was assigned as 24*Z*. The skeletal ^{1}H signals of **5a** were consistent with the corresponding signals of Δ^7-sten-3β-yl acetates.[6)] The combined evidence confirmed that **5a** was (22*E*,24*Z*)-5α-stigmasta-7,22,24(24^1)-trien-3β-yl acetate.[18)]

The molecular formulae of **6a** ($C_{31}H_{50}O_2$; M^+, *m/z* 454.3791) and **7a** ($C_{31}H_{48}O_2$; M^+, *m/z* 452.3674) were determined by HR-MS. This suggested that these are the acetates of C_{29} sterols, **6a** having two degrees of unsaturation and **7a** having three degrees of unsaturation. Acetate **6a** was shown to have a saturated C_{10} side chain[8)] and a di-unsaturated skeleton by the presence of prominent ions at *m/z* 313 [M^+ − s.c.($C_{10}H_{21}$)] and 253 (313 − HOAc) in the MS. The MS of **7a** exhibited significant fragments at *m/z* 354 [$M^+ - C_7H_{14}$ (part of s.c.)] and 311 (M^+ − s.c. − 2H), indicating that this has a C-24 mono-unsaturated C_{10} side chain and a di-unsaturated skeleton.[8)] Both **6a** and **7a** were $\Delta^{7,9(11)}$-dienes since they showed essentially the same UV absorption spectrum as **4a** and the relevant ^{1}H signals (H-7, H-11, H-18, H-19) of these acetates (Table 1) were consistent with those of the corresponding signals of 5α-ergosta-7,9(11),24(24^1)-trien-3β-ol.[19)] The side chain ^{1}H signals of **6a** (Table 1) were as expected for a sterol possessing a stigmastane (24*R*/α-ethyl substituted) side chain.[6)] Thus **6a** was 5α-stigmasta-7,9(11)-dien-3β-yl acetate. Consistency among the side chain ^{1}H signals of **7a** (Table 1) and those of **2a**, **3a**, and **4a** (*vide supra*), in combination with our conclusions about the structure of its skeleton, implied that **7a** is the (24*E*)-5α-stigmasta-7,9(11),24(24^1)-trien-3β-yl acetate.

The MS of **8a** showed M^+ at *m/z* 468.3909 ($C_{32}H_{52}O_2$), along with prominent fragment ions at *m/z* 370 [$M^+ - C_7H_{14}$ (part of side chain)], 327 [M^+ − s.c.($C_{10}H_{19}$) − 2H], 287 [M^+ − s.c. − 42 (ring D)], and 273 (287 − CH_2). This indicated that **8a** was an acetate of a C_{30} sterol with two degrees of unsaturation, one at C-24 in the C_{10} side chain and the other in the skeleton with an additional methyl group at C-14.[8)] The skeletal ^{1}H signals (Table 1) of **8a** were consistent with the corresponding signals of synthetic (24*R*+*S*)-14α,24-dimethyl-5α-cholest-7-en-3β-yl acetate,[20)] whereas the side chain ^{1}H signals were consistent with those of **2a**, **3a**, **4a**, and **7a**. Thus **8a** was (24*E*)-14α-methyl-5α-stigmasta-7,24(24^1)-dien-3β-yl acetate.

This is the first report of the natural occurrence of 4,4-dimethyl-Δ^7-cholestene-, 4α,14α-dimethyl-Δ^7-cholestene (29-nor-Δ^7-lanostene)-, and 14α-methyl-Δ^7-cholestene-skeletons. Several 4α,14α-dimethyl-$\Delta^{9(11)}$-cholestene (or 29-nor-$\Delta^{9(11)}$-lanostene)-, 4α,14α-dimethyl-$\Delta^{7,9(11)}$-cholestadiene (or 29-nor-$\Delta^{7,9(11)}$-lanostadiene)-, and $\Delta^{7,9(11)}$-cholestadiene-type sterols have been reported to occur in some higher plants.[21)] Also, **5** is the first example of a naturally occurring sterol with a (22*E*,24*Z*)-$\Delta^{22,24(24^1)}$-stigmastadiene-type side chain isolated from a natural source. (22*E*,24*Z*)-Stigmasta-5,22,24(24^1)-trien-

3β-ol, has been synthesized.[17a] Some nematodes have been reported to contain (22*E*,24*E*)-stigmasta-5,22,24(24[1])-trien-3β-ol {a metabolite of stigmasterol [(22*E*)-stigmasta-5,22-dien-3β-ol]}.[17]

Abundances and chromatographic data of all (=53) identified sterols and triterpene alcohols from this source

Table 2. Abundance (%) and Chromatographic Data of Acetates of Triterpene Alcohols, 4α-Methylsterols, and Sterols from the Saponified Neutral Fraction of the *Bryonia dioica* Root Extract

Compound[a]	Abundance (%)[b]	Acetate Rt_R[c] (HPLC)	Acetate Rt_R[c] (GLC)
Triterpene alcohol fraction (fr. A)			
Cycloart-24-enol (cycloartenol)	39.2	1.01	1.82
24-Methylcycloart-24(24[1])-enol (24-methylenecycloartanol)	5.7	1.09	2.00
24-Methyllanosta-8,24(24[1])-dienol	2.0	1.00	1.72
Lanosta-9(11),24-dienol (parkeol)	2.0	0.90	1.77
24-Methyllanosta-9(11),24(24[1])-dienol	1.9	0.99	1.94
24-Methyllanosta-7,9(11),24(24[1])-trienol	1.1	0.74	1.72
Eupha-7,24-dienol (butyrospermol)	3.5	0.87	1.66
Tirucall-5-en-3β-ol	0.1	0.92	1.35
Tirucalla-5,24-dien-3β-ol	1.0	0.74	1.64
24-Methyltirucalla-5,24(24[1])-dien-3β-ol	0.2	0.80	1.81
(24*S*)-24-Methyltirucalla-5,25-dien-3β-ol	0.2	0.79	1.77
Tirucalla-7,24-dienol	1.9	0.93	1.90
Dammara-20,24-dienol (dammaradienol)	2.0	0.69	1.67
24-Methyldammara-20,24(24[1])-dienol (24-methylenedammarenol)	3.0	0.75	1.78
10α-Cucurbita-5,24-dienol (10α-cucurbitadienol)	5.8	0.71	1.49
4,4-Dimethylporiferasta-7,25-dienol(24*S*/β) (**1**)[d]	0.7	1.08	2.39
Urs12-enol (α-amyrin)	0.8	1.06	1.91
Olean-12-enol (β-amyrin)	3.2	0.97	1.69
D:C-Friedoolean-7-enol (multiflorenol)	0.5	1.19	2.17
D:C-Friedoolean-8-enol (isomultiflorenol)	5.5	1.00	1.76
D:C-Friedours-8-enol (isobauerenol)	1.6	1.13	1.76
D-Friedoolean-14-enol (taraxerol)	0.8	0.94	1.64
Taraxast-20-enol (ψ-taraxasterol)	1.0	1.12	2.46
Lup-20(29)-enol (lupeol)	3.0	0.79	1.99
Others, unidentified	13.3		
4α-Methylsterol fraction (fr. B)			
4α-Methylergosta-7,24(24[1])-dienol (gramisterol)	2.2	0.97	1.71
(24*Z*)-4α-Methylstigmasta-7,24(24[1])-dienol (citrostadienol)	2.4	1.21	2.22
(24*E*)-4α-Methylstigmasta-7,24(24[1])-dienol (isocitrostadienol)	30.0	1.18	2.12
24-Methyl-29-norcycloart-24(24[1])-enol (cycloeucalenol)	8.7	0.96	1.72
24-Methyl-29-norlanosta-8,24(24[1])-dienol (obtusifoliol)	3.5	0.84	1.46
(24*E*)-24-Ethyl-29-norlanosta-7,24(24[1])-dienol (**2**)[d]	24.2	1.07	2.17
24-Methyl-29-norlanosta-9(11),24(24[1])-dienol	1.1	0.84	1.66
(24*E*)-24-Ethyl-29-norlanosta-9(11),24(24[1])-dienol (**3**)[d]	0.7	1.05	2.04
(24*Z*)-24-Ethyl-29-norlanosta-9(11),24(24[1])-dienol	2.7	1.07	2.14
(24*E*)-24-Ethyl-29-norlanosta-7,9(11),24(24[1])-trienol (**4**)[d]	6.3	0.79	1.83
Others, unidentified	18.2		
Sterol fraction (fr. C)			
Cholest-5-enol (cholesterol)	0.2	1.00	1.00
Campest-7-enol (24*R*/α)	1.4	1.11	1.50
Ergosta-7,24(24[1])-dienol (episterol)	0.3	0.80	1.57
Ergosta-7,25-dienol (24*S*/β)	0.5	0.79	1.54
Stigmast-7-enol (schottenol) (24*R*/α)[e]	20.4	1.26	1.81
Poriferast-7-enol (22-dihydrochondrillasterol) (24*S*/β)[e]	2.5	1.26	1.80
Stigmasta-7,22-dienol (spinasterol) (24*S*/α)[e]	5.9	1.09	1.62
Poriferasta-7,22-dienol (chondrillasterol) (24*R*/β)[e]	1.5	1.09	1.62
Stigmasta-7,25-dienol (24*R*/α)	2.1	0.96	1.81
Poriferasta-7,25-dienol (24*S*/β)	0.5	0.94	1.84
Poriferasta-7,22,25-trienol (24*S*/β)	1.2	0.76	1.70
(24*Z*)-Stigmasta-7,24(24[1])-dienol (avenasterol)	1.2	1.01	2.01
(24*E*)-Stigmasta-7,24(24[1])-dienol (isoavenasterol)	53.5	0.98	1.93
(22*E*,24*Z*)-Stigmasta-7,22,24(24[1])-trienol (**5**)[d]	0.9	0.75	1.92
Stigmast-9(11)-enol (24*R*/α)[e]	0.5	1.13	1.46
Poriferast-9(11)-enol (24*S*/β)[e]	0.2	1.13	1.46
Stigmasta-7,9(11)-dienol (24*R*/α) (**6**)[d]	0.9	0.97	1.73
(24*E*)-Stigmasta-7,9(11),24(24[1])-trienol (**7**)[d]	3.1	0.78	1.88
(24*E*)-14α-Methylstigmasta-7,24(24[1])-dienol (**8**)[d]	0.9	1.01	1.96
Others, unidentified	2.3		

a) All compounds have a hydroxyl group at C-3β. All compounds, with the exception of C-5 unsaturated sterols, are 5α-compounds. All C-22–C-23 double bonds are *trans* (*E*) oriented. *b*) Abundance in each fraction determined based on the HPLC and GLC data. *c*) Standard: cholesteryl acetate (Rt_R: 1.00; actual retention times: 48 min in HPLC and 24 min in GLC). *d*) New compounds reported in this paper. *e*) Isolated as the C-24 epimeric mixture.

are given in Table 2. They include four triterpene alcohols recently reported in another paper.[22] Identification of known compounds was made by chromatographic (HPLC, GLC) and spectral (^{1}H-NMR, MS) comparison with corresponding authentic materials. ^{1}H-NMR data of the acetates of the eight novel sterols are listed in Table 1. The ^{13}C-NMR data of **2a**, **4a**, and **5a** are included in the Experimental Section.

This paper concludes our work on sterols and triterpenes from the roots of *B. dioica*.

Experimental

Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. All sterols and their acetyl derivatives were crystallized from acetone–MeOH. Argentic TLC plates [silica gel–$AgNO_3$ (4:1, w/w)] were developed two times with CCl_4–CH_2Cl_2 (4:1). Preparative HPLC was carried out on an octadecyl silica (ODS) column (Ultrasphere ODS column, 5 μ, 25 cm × 10 mm i.d.; Beckman Instruments, Inc., San Ramon, California) with MeOH (4 ml/min) using an SSC Flow System 3100K (Senshu Scientific Co., Tokyo) and an ERC-7520 refractive index detector (Erma Optical Works, Ltd., Tokyo). For purification purposes, two other ODS columns (25 cm × 10 mm i.d.), a Superiorex ODS (S-5 μm) column (Shiseido Co., Tokyo) and a TSK ODS-120A 5 μ column (Toso Co., Tokyo), were used, when necessarily, under the same operating conditions as described above. Gas–liquid chromatography (GLC) was run on a Shimadzu GC-14A apparatus using a DB-17 fused silica capillary column (30 m × 0.3 mm i.d., column temp. 275 °C). In both HPLC (Ultrasphere ODS column) and GLC, cholesteryl acetate was the standard for the determination of R t_R for the acetyl derivatives of sterols. UV spectra were determined on a Shimadzu UV-300 spectrometer in EtOH. Electron-impact MS and HR-MS were obtained using a Hitachi M-80B double focusing gas chromatograph-mass spectrometer (70 eV) using the direct inlet system. NMR spectra were recorded by JEOL GX-400 and GSX-400 spectrometers at 400 MHz (^{1}H-NMR) and 100.62 MHz (^{13}C-NMR) in $CDCl_3$ with tetramethylsilane (^{1}H-NMR) and $CDCl_3$ at δ 77.0 (^{13}C-NMR) as internal standards, and chemical shifts were recorded in δ values. Acetylation (Ac_2O–pyridine) and hydrolysis of acetates (5% KOH in MeOH) were performed at room temperature overnight. Sources of reference triterpene alcohols and sterols are given in our recent review article.[21] The roots of *Bryonia dioica* JACQ. were collected in The Netherlands in September, 1986.

Signal assignment of ^{13}C-NMR spectra was made by comparison with literature data of relevant compounds,[23] and using the results of the following NMR experiments: ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H shift correlated spectroscopy (COSY), ^{1}H–^{13}C COSY, difference NOE, and heteronuclear multiple-bond correlation (HMBC) experiments.

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 extract (500 g) by alkaline hydrolysis (5% KOH in MeOH, reflux, 3 h). The neutral lipids were chromatographed over a silica gel (250 g) column with hexane, hexane–ethyl acetate (EtOAc) (9:1, v/v), and hexane–EtOAc (4:1) as eluants. The hexane–EtOAc (9:1) eluted a fraction, which after rechromatography over silica gel column yielded a triterpene alcohol (376 mg) and 4α-methylsterol (274 mg) fractions. The hexane–EtOAc (4:1) eluted a fraction which after rechromatography gave a sterol fraction (2880 mg). Acetylation of individual fractions yielded acetylated fractions of triterpene alcohol (374 mg), 4α-methylsterol (209 mg), and sterol (2459 mg). Isolation of individual components from the acetylated fractions was performed by argentic TLC followed by HPLC. D:C-*friedo*-urs-8-en-3β-ol (isobauerenol) has so far been isolated only from *Evodia fraxinifolia* (Rutaceae)[24] but no NMR data have been reported. For that reason, assigned ^{1}H- and ^{13}C-NMR data of D:C-*friedo*-urs-8-en-3β-yl (isobauerenyl) acetate which we isolated from the acetylated triterpene alcohol fraction of *B. dioica* root extract, are included in this paper. *B. dioica* is dioecious. However, the sterol/triterpene composition of the roots of male and female plants was the same.

(24*S*)-4,4-Dimethyl-5α-poriferasta-7,25-dien-3β-yl Acetate (1a) and (24*S*)-4,4-Dimethyl-5α-poriferasta-7,25-dien-3β-ol (1) **1a**: MS *m/z* (%): 482 (M^+, 21), 467 (12), 422 (2), 407 (3), 369 (2), 341 (38), 301 (3), 283 (13), 281 (3), 257 (4), 255 (5), 241 (9), 43 (100). HR-MS *m/z*: 482.4126 [Calcd for $C_{33}H_{54}O_2$ (M^+): 482.4121]; 407.3692 (Calcd for $C_{30}H_{47}$: 407.3675); 341.2474 (Calcd for $C_{23}H_{33}O_2$: 341.2479); 301.2188 (Calcd for $C_{20}H_{29}O_2$: 301.2166); 283.2414 (Calcd for $C_{21}H_{31}$: 283.2424); 281.2268 (Calcd for $C_{21}H_{29}$: 281.2268); 241.1958 (Calcd for $C_{18}H_{25}$: 241.1955). Alkaline hydrolysis of **1a** yielded a free sterol **1**. **1**: MS *m/z* (%): 440 (M^+, 18), 425 (9), 422 (2), 407 (3), 299 (29), 283 (9), 281 (2), 273 (4), 259 (3), 257 (4), 255 (4), 241 (5), 55 (100). HR-MS: *m/z* 440.4035 [Calcd for $C_{31}H_{52}O$ (M^+): 440.4016]. ^{1}H-NMR δ: 0.51 (3H, s, H-18), 0.80 (3H, t, J=7.3 Hz, H-24^2), 0.86 (3H, s, H-29), 0.90 (3H, s, H-19), 0.91 (3H, d, J=6.6 Hz, H-21), 0.99 (3H, s, H-28), 1.56 (3H, br s, H-26), 3.25 (1H, dd like, J=4.5, 9.6 Hz, H-3α), 4.64 (1H, br d, J=2.5 Hz), 4.73 (1H, dt, J=2.5, 1.4 Hz) (H-27).

(24*E*)-24-Ethyl-29-nor-5α-lanosta-7,24(24^1)-dien-3β-yl Acetate (2a) and (24*E*)-24-Ethyl-29-nor-5α-lanosta-7,24(24^1)-dien-3β-ol (2) **2a**: mp 101—102 °C. MS *m/z* (%): 482 (M^+, 9), 467 (32), 422 (1), 407 (19), 384 (21), 369 (22), 355 (3), 341 (4), 327 (2), 316 (5), 309 (11), 301 (6), 287 (4), 276 (15), 256 (13), 241 (13), 227 (5), 215 (10), 201 (7), 55 (100). HR-MS: *m/z* 482.4106 [Calcd for $C_{33}H_{54}O_2$ (M^+): 482.4121]; 384.3020 (Calcd for $C_{26}H_{40}O_2$: 384.3026); 369.2744 (Calcd for $C_{25}H_{37}O_2$: 369.2792); 341.2519 (Calcd for $C_{23}H_{33}O_2$: 341.2479); 301.2164 (Calcd for $C_{20}H_{29}O_2$: 301.2166); 287.2025 (Calcd for $C_{19}H_{27}O_2$: 287.2009). ^{13}C-NMR δ (assignment): 13.2 (C-24^2), 13.4 (C-19), 15.1 (C-28), 16.1 (C-18), 19.0 (C-21), 20.4 (C-11), 21.4 (CO<u>Me</u>), 22.1 (C-26 or C-27), 22.2 (C-27 or C-26), 24.8 (C-30), 26.0 (C-23), 26.8 (C-6), 27.2 (C-2), 27.6 (C-16), 32.2 (2 × C, C-12, C-15), 34.8 (C-25), 34.9 (C-10), 35.6 (C-22), 36.7 (C-1), 37.0 (C-20), 37.1 (C-4), 44.4 (C-13), 44.7 (C-9), 46.8 (C-5), 50.5 (C-17), 52.1 (C-14), 78.5 (C-3), 115.6 (C-24^1), 116.0 (C-7), 145.3 (C-8), 147.0 (C-24), 170.9 (<u>CO</u>Me). Alkaline hydrolysis of **2a** yielded a free sterol **2**. **2**: mp 161—162 °C. MS *m/z* (%): 440 (M^+, 5), 425 (27), 407 (4), 342 (17), 327 (23), 313 (3), 274 (6), 259 (9), 256 (6), 245 (3), 241 (7), 234 (12), 215 (5), 55 (100). HR-MS: *m/z* 440.4034 [Calcd for $C_{31}H_{52}O$ (M^+): 440.4016]. ^{1}H-NMR: δ 0.67 (3H, s, H-18), 0.85 (3H, s, H-19), 0.96 (3H, d, J=6.2 Hz, H-21), 0.98 (3H, s, H-30), 0.98 (3H, d, J=6.6 Hz, H-28), 0.99 (6H, d, J=6.6 Hz, H-26, H-27), 1.58 (3H, d, J=6.7 Hz, H-24^2), 2.23 (1H, sept., J=6.3 Hz, H-25), 3.12 (1H, dt, J=4.8, 11.2 Hz, H-3α), 5.17 (1H, m, H-7), 5.19 (1H, q, J=6.6 Hz, H-24^1).

(24*E*)-24-Ethyl-29-nor-5α-lanosta-9(11),24(24^1)-dien-3β-yl Acetate (3a) mp 126—128 °C. MS *m/z* (%): 482 (M^+, 8), 467 (23), 439 (3), 422 (1), 407 (13), 384 (28), 369 (6), 355 (2), 341 (22), 324 (2), 309 (7), 301 (14), 287 (5), 275 (4), 274 (4), 241 (15), 227 (6), 215 (6), 201 (9), 55 (100). HR-MS: *m/z* 482.4140 [Calcd for $C_{33}H_{54}O2$ (M^+): 482.4121]; 384.3040 (Calcd for $C_{26}H_{40}O_2$: 384.3026); 369.2760 (Calcd for $C_{25}H_{37}O_2$: 369.2792); 341.2498 (Calcd for $C_{23}H_{33}O_2$: 341.2479); 301.2174 (Calcd for $C_{20}H_{29}O_2$: 301.2166); 287.1976 (Calcd for $C_{19}H_{27}O_2$: 287.2009).

(24*E*)-24-Ethyl-29-nor-5α-lanosta-7,9(11),24(24^1)-trien-3β-yl Acetate (4a) mp 140—142 °C. UV λ_{max} nm: 233, 242, 250. MS *m/z* (%): 480 (M^+, 65), 465 (14), 420 (4), 405 (14), 382 (13), 367 (9), 339 (30), 314 (6), 299 (21), 286 (9), 285 (8), 281 (7), 274 (11), 259 (4), 253 (3), 239 (37), 226 (27), 225 (17), 213 (13), 55 (100). HR-MS: *m/z* 480.3944 [Calcd for $C_{33}H_{52}O_2$ (M^+): 480.3963]; 382.2855 (Calcd for $C_{26}H_{38}O_2$: 382.2870); 367.2686 (Calcd for $C_{25}H_{35}O_2$: 367.2635); 339.2319 (Calcd for $C_{23}H_{31}O_2$: 339.2322); 299.1987 (Calcd for $C_{20}H_{27}O_2$: 299.2010); 285.1907 (Calcd for $C_{19}H_{25}O_2$: 285.1853). ^{13}C-NMR δ (assignment): 13.2 (C-24^2), 15.1 (C-28), 15.7 (C-18), 18.5 (C-21), 20.6 (C-19), 21.3 (CO<u>Me</u>), 22.1 (C-26 or C-27), 22.2 (C-27or C-26), 24.4 (C-1), 25.6 (C-30), 26.0 (C-23), 26.4 (C-6), 27.4 (C-2), 27.9 (C-16), 31.5 (C-15), 34.8 (C-25), 35.5 (C-22), 36.3 (C-10), 36.6 (C-4), 36.9 (C-20), 37.9 (C-12), 43.8 (C-13), 45.6 (C-5), 50.4 (C-14), 50.7 (C-17), 78.3 (C-3), 115.6 (C-24^1), 117.4 (C-11), 119.0 (C-7), 143.1 (2 × C, C-8, C-9), 147.0 (C-24), 171.0 (<u>CO</u>Me).

(22*E*,24*Z*)-5α-Stigmasta-7,22,24(24^1)-trien-3β-yl Acetate (5a) and (22*E*,24*Z*)-5α-Stigmasta-7,22,24(24^1)-trien-3β-ol (5) **5a**: mp 102—104 °C. UV λ_{max} nm: 238. NOE experiment: irradiation of H-24^2 (δ 1.71, d, J=7.2 Hz) resulted in appreciable enhancement of H-23 (δ 6.17, d, J=15.7 Hz); irradiation of H-24^1 (δ 5.32, q, J=6.9 Hz) enhanced H-25 (δ 2.66, sept., J=6.6 Hz) and H-26/H-27 (δ 1.02, d, J=6.6 Hz and δ 1.02, d, J=6.9 Hz). This meant that C-24^2 was oriented toward C-23 (*cis*-orientation). MS *m/z* (%): 452 (M^+, 5), 437 (1), 409 (1), 342 (31), 327 (6), 313 (100), 282 (3), 255 (12), 253 (5), 227 (3), 213 (7), 199 (4). HR-MS: *m/z* 452.3678 [Calcd for $C_{31}H_{48}O_2$ (M^+): 452.3652]; 409.3138 (Calcd for $C_{28}H_{41}O_2$: 409.3104); 342.2558 (Calcd for $C_{23}H_{34}O_2$: 342.2557); 327.2291 (Calcd for $C_{22}H_{31}O_2$: 327.2322); 313.2198 (Calcd for $C_{21}H_{29}O_2$: 313.2166); 282.2374 (Calcd for $C_{21}H_{30}$: 282.2346).

^{13}C-NMR δ (assignment): 12.2 (C-18), 13.0 (C-19), 13.3 (C-24^2), 21.0 (C-21), 21.5 (2×C, COMe, C-11), 22.2 (C-26 or C-27), 22.6 (C-27 or C-26), 22.9 (C-15), 27.5 (C-2), 28.2 (C-16), 29.5 (C-6), 30.2 (C-25), 33.8 (C-4), 34.2 (C-10), 36.8 (C-1), 39.4 (C-12), 40.0 (C-5), 41.3 (C-20), 43.4 (C-13), 49.3 (C-9), 55.0 (C-14), 56.0 (C-17), 73.5 (C-3), 117.4 (C-7), 118.0 (C-24^1), 123.5 (C-23), 136.4 (C-22), 139.4 (C-8), 143.3 (C-24), 170.7 (COMe). Alkaline hydrolysis of **5a** gave a free sterol **5**. **5**: MS *m/z* (%): 410 (M^+, 4), 395 (2), 300 (32), 285 (8), 271 (100), 255 (8), 227 (3), 213 (3), 199 (2). HR-MS: *m/z* 410.3560 [Calcd for $C_{29}H_{46}O$ (M^+): 410.3547]; 300.2456 (Calcd for $C_{21}H_{32}O$: 300.2452); 285.2257 (Calcd for $C_{20}H_{29}O$: 285.2217). ^{1}H-NMR: δ 0.58 (3H, s, H-18), 0.81 (3H, s, H-19), 1.02 (3H, d, J=6.6 Hz), 1.03 (3H, d, J=6.9 Hz) (H-26, H-27), 1.08 (3H, d, J=6.6 Hz, H-21), 1.71 (3H, d, J=7.1 Hz, H-24^2), 2.54 (1H, sept., J=6.9 Hz, H-25), 3.59 (1H, tt, J=4.6, 11.2 Hz, H-3α), 5.17 (1H, m, H-7), 5.32 (1H, q, J=6.9 Hz, H-24^1), 5.51 (1H, dd, J=8.8, 15.7 Hz, H-22), 6.17 (1H, d, J=15.7 Hz, H-23).

5α-Stigmasta-7,9(11)-dien-3β-yl Acetate (6a) mp 159—162 °C. UV λ_{max} nm: 233, 240, 250. MS *m/z* (%): 454 (M^+, 82), 439 (11), 394 (11), 379 (17), 341 (3), 313 (19), 311 (3), 300 (4), 286 (9), 271 (7), 259 (15), 253 (17), 246 (7), 226 (5), 211 (26), 43 (100). HR-MS: *m/z* 454.3791 [Calcd for $C_{31}H_{50}O_2$ (M^+): 454.3807]; 313.2194 (Calcd for $C_{21}H_{39}O_2$: 323.2948); 253.1962 (Calcd for $C_{19}H_{25}$: 253.1955).

(24*E*)-5α-Stigmasta-7,9(11),24(24^1)-trien-3β-yl Acetate (7a) mp 113—115 °C. UV λ_{max} nm: 235, 242, 250. MS *m/z* (%): 452 (M^+, 19), 437 (1), 409 (1), 392 (1), 377 (3), 354 (4), 339 (3), 311 (73), 285 (19), 279 (3), 270 (4), 259 (4), 253 (6), 251 (7), 237 (3), 225 (3), 211 (17), 55 (100). HR-MS: *m/z* 452.3674 [Calcd for $C_{31}H_{48}O_2$ (M^+): 452.3651]; 354.2588 (Calcd for $C_{24}H_{34}O_2$: 354.2557); 311.1960 (Calcd for $C_{21}H_{27}O_2$: 311.2010).

(24*E*)-14α-Methyl-5α-stigmasta-7,24(24^1)-dien-3β-yl Acetate (8a) MS *m/z* (%): 468 (M^+, 1), 453 (8), 393 (3), 370 (11), 355 (10), 341 (2), 327 (2), 313 (9), 295 (4), 287 (6), 273 (3), 262 (8), 255 (5), 242 (7), 227 (9), 201 (6), 55 (100). HR-MS: *m/z* 468.3909 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3963]; 370.2826 (Calcd for $C_{25}H_{38}O_2$: 370.2870); 327.2296 (Calcd for $C_{22}H_{31}O_2$: 327.2322); 287.1018 (Calcd for $C_{19}H_{27}O_2$: 287.2010); 273.1877 (Calcd for $C_{18}H_{25}O_2$: 273.1853).

D: C-*friedo*-Urs-8-en-3β-yl (Isobauerenyl) Acetate mp 229—231 °C (lit.[24] 231—233 °C). ^{1}H-NMR: δ 0.84 (3H, s, H-27), 0.87 (3H, s, H-24), 0.88 (3H, s, H-23), 0.90 (3H, d, J=6.1 Hz, H-30), 0.98 (3H, s, H-25), 0.99 (3H, d, J=7.1 Hz, H-29), 1.00 (3H, s, H-26), 1.05 (3H, s, H-28), 2.05 (3H, s, OAc-3β), 4.50 (1H, dd, J=4.7, 11.5 Hz, H-3α). ^{13}C-NMR δ (assignment): 15.6 (C-27), 16.6 (C-24), 19.0 (C-6), 20.0 (C-25), 20.6 (C-11), 21.3 (COMe), 22.2 (C-26), 22.4 (C-30), 24.2 (C-2), 25.2 (C-29), 25.3 (C-15), 27.3 (C-7), 28.0 (C-23), 29.1 (C-13), 29.6 (C-21), 31.8 (C-17), 31.9 (C-19), 33.1 (C-22), 34.7 (C-1), 36.0 (C-20), 37.4 (C-10), 37.8 (C-4), 37.9 (C-16), 38.2 (2×C, C-13, C-28), 41.1 (C-14), 50.6 (C-5), 52.4 (C-18), 81.0 (C-3), 134.0 (C-9), 134.6 (C-8), 171.1 (COMe).

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