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PHYTOCHEMISTRY

(20*R*,23*E*)-Eupha-8,23-diene-3β,25-diol from *Tripetalum cymosum*

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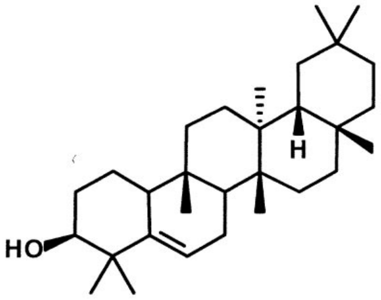
Abstract

A new euphane triterpenoid, (20*R*,23*E*)-eupha-8,23-diene-3β,25-diol, was isolated from the leaves of *Tripetalum cymosum* and its structure was determined by spectral analysis and chemical correlation with euphol. Caryophyllene oxide, friedelin, euphol and putranjivic acid were also obtained from the leaves whilst the bark afforded euphol, glutin-5-en-3β-ol, butyrospermol and dammara-20,24-dien-3β-ol. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Tripetalum cymosum; Guttiferae; Triterpenoids; Euphane; (20*R*,23*E*)-eupha-8,23-diene-3β,25-diol

1. Introduction

Members of the Guttiferae are well known sources of aromatic metabolites such as xanthones (Bennett, & Lee, 1989; Peres, & Nagem, 1977) and coumarins (McKee, Fuller, Covington, Cardellina et al., 1996). Whilst many such compounds have been reported from large genera (>100 species) such as *Garcinia* (Harrison, Leong, Leong, Sia, Sim et al., 1994), *Calophyllum* (Iinuma, Ito, Tosa, Tanaka et al., 1997), *Clusia* (Henry, Jacobs, Carrington, McLean, & Reynolds, 1996) and *Hypericum* (Ishiguro, Nagareya, Suitani, & Fukumoto, 1997), the Guttiferae also contains a number of small genera, e.g., *Archytaea* (Kubitzki, Lins Mesquita, & Gottlieb, 1978), *Pentadesma* (Gunasekera, Sivapalan, Sultanbawa, & Ollis, 1977) and *Ploiarium* (Bennett, Lee, Lowrey, 1990; Bennett, Harrison, Sia, Sim, & Connolly, 1992; Bennett, Harrison, Lim, Sim et al., 1991), which contain fewer than 10 species each (Willis, 1973). One such genus is *Tripetalum*, the sole member of which, *T. cymosum* K. Schum., is native to Papua New Guinea where it is cultivated by the natives who use the juice from the fruits to stain their teeth black (Usher, 1984). There are no previous reports concerning the constituents of this plant. We have now studied the hexane extracts of both bark and leaves and found them to lack the aromatic compounds which are typical of the Guttiferae.



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2. Results and discussion

Chromatographic separation of the hexane extract of the bark gave the known triterpenoids glutin-5-en-3β-ol (**1**) (Kitajima, Arai, & Tanaka, 1994; Carvalho, & Seita, 1993; Mahato, Das, & Sahu, 1981), euphol (**2**) (De Pascual Teresa, Urones, Marcos, Basabe et al., 1987; Gewali, Hattori, Tezuka, Kikuchi, & Namba, 1990), butyrospermol (eupha-7,24-dien-3β-ol) (**3**) (De Pascual Teresa et al., 1987) and dammara-20,24-dien-3β-ol (**4**) (Mills, & Werner, 1955) which were identified by comparison of their physical properties with literature values. As euphol is difficult to distinguish from its C-20 epimer tirucallol, its identity was con-

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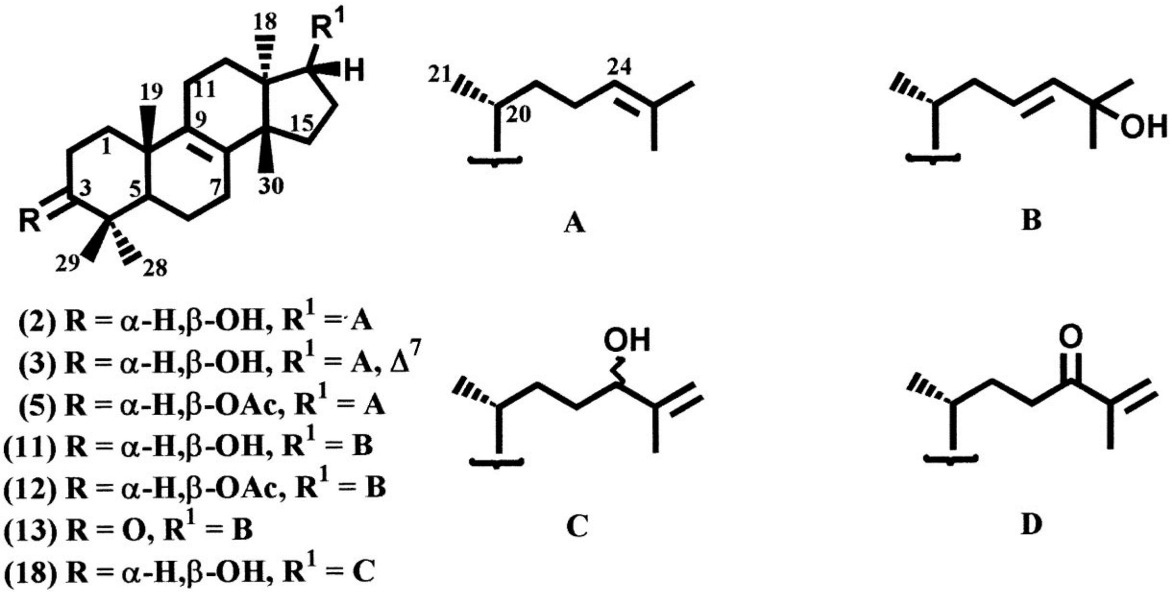


(2) R = α-H,β-OH, R^1 = A
(3) R = α-H,β-OH, R^1 = A, Δ^7
(5) R = α-H,β-OAc, R^1 = A
(11) R = α-H,β-OH, R^1 = B
(12) R = α-H,β-OAc, R^1 = B
(13) R = O, R^1 = B
(18) R = α-H,β-OH, R^1 = C

firmed by conversion to the acetate (**5**) (De Pascual Teresa et al., 1987). The ^{13}C NMR spectrum of dammara-20,24-dien-3β-ol (see Table 1) has not previously been reported. The present assignments were made by comparison with those of dammarenediol II (**6**) (Tori, Matsuda, Sono, & Asakawa, 1988).

Euphol has previously been isolated from *Garcinia myrtifolia* (Guttiferae) (Mills & Werner, 1955) and several *Euphorbia* species (Euphorbiaceae) (De Pascual Teresa, Urones, Marcos, Basabe et al., 1987; Gewali, Hattori, Tezuka, Kikuchi, & Namba, 1990). Its ^{13}C NMR signals were recently completely assigned by 2D NMR (Gewali et al., 1990; (Spino, Lal, Sotheeswaran & Aalbersberg, 1995) and some of the previous assignments (De Pascual Teresa et al., 1987) were revised. Glutin-5-en-3β-ol has been previously isolated from *Mammea acuminata* (Guttiferae) (Bandaranayake, Karunanayake, Sotheeswaran, & Sultanbawa, 1980) as well as from several *Euphorbia* species (Fisher, & Seiler, 1961; Starratt, 1966) and *Securinega tinctoria* (Euphorbiaceae) (Carvalho, & Seita, 1993). Plants from a variety of families, e.g., Gramineae (Akihisa, Yamamoto, Tamura, Kimura, et al., 1992), Moraceae (Kitajima, Arai, & Tanaka, 1994), Scrophulariaceae (Mahato, Das, & Sahu, 1981), Celastraceae (Gonzalez, Ferro, & Ravelo, 1987) and Verbenaceae (Lin, Kuo, & Che, 1989) are also sources of this compound. Butyrospermol (**3**) has been isolated from a number of sources, one being the herbaceous shrub *Euphorbia broteri* (Euphorbiaceae) (De Pascual Teresa, 1987). Dammara-20,24-dien-3β-ol (**4**), which was first found in the dammar resins from trees of the Dipterocarpaceae family (Mills, & Werner, 1955), was also isolated from *Vismia guaramirangae* (Camele,

Table 1
^{13}C NMR Shifts for Dammara-20,24-dien-3β-ol (**4**) and Dammarenediol II (**6**) [21].

Carbon	**4**[a]	**6**[b]
1	39.1	39.0
2	27.1	27.4
3	79.0	78.9
4	39.0	39.0
5	56.0	55.9
6	18.3	18.3
7	35.5	35.3
8	40.5	40.4
9	51.0	50.7
10	37.3	37.1
11	21.4	21.6
12	27.5	27.6
13	45.3	42.3
14	49.5	50.3
15	31.4	31.2
16	25.0	24.9
17	47.9	49.9
18	15.7	15.5
19	16.0	16.2
20	152.8	75.4
21	107.5	25.4
22	39.1	40.5
23	28.9	22.6
24	124.5	124.8
25	131.4	131.5
26	25.7	25.7
27	17.7	17.7
28	28.0	28.0
29	15.4	15.4
30	16.2	16.5

In $CDCl_3$. [a] 125 MHz, [b] 100 MHz.

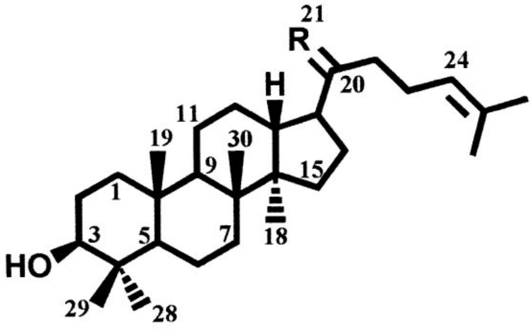
Table 2
^{13}C NMR Shifts for Friedelin (**8**) [26], Putranjivic Acid (**9**) and Methyl Putranjivate (**10**).

Carbon	8^{a}	9^{b}	10^{b}
1	22.3	21.2	21.4
2	41.5	41.6	41.6
3	213.2	179.5	174.3
4	58.2	151.0	151.0
5	42.1	42.1	42.1
6	41.3	37.2	37.3
7	18.2	18.0	18.0
8	53.1	53.1	53.1
9	37.4	38.4	38.4
10	59.4	58.3	58.4
11	35.6	35.26	35.3
12	30.5	30.3	30.3
13	39.7	39.7	39.7
14	38.3	38.7	38.7
15	32.4	32.3	32.3
16	36.0	36.1	36.1
17	30.0	30.0	30.0
18	42.8	42.9	42.9
19	35.3	35.34	35.2
20	28.1	28.2	28.2
21	32.7	32.9	32.8
22	39.2	39.3	39.3
23	6.8	111.0	110.8
24	14.6	18.0	18.0
25	17.9	18.2	18.2
26	20.2	18.8	18.8
27	18.6	20.2	20.2
28	32.1	31.9	31.8
29	35.0	32.1	32.1
30	31.8	35.0	35.0
OCH_3	–	–	51.4

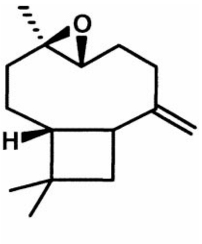
Delle Monache, Delle Monache, Marini Bettolo, & Alves De Lima, 1982) and *V. martiana* (Guttiferae) (Nagem, & Faria, 1990).

The leaf extract was also subjected to extensive chromatographic separation to give the known compounds caryophyllene oxide (**7**) (Heymann, Tezuka, Kikuchi, & Supriyatna, 1994), friedelin (**8**), euphol (**1**) (De Pascual Teresa et al., 1987; Gewali et al., 1990) and putranjivic acid (**9**) (Chopra, Jain, & Seshadri, 1969; Aoyagi, Tsuyuki, & Takahashi, 1973) which were identified by comparison of their physical properties with those in the literature or, in the case of friedelin, by comparison with an authentic sample. The ^{13}C NMR data for putranjivic acid and its methyl ester (**10**) (Chopra, Jain, & Seshadri, 1969; Aoyagi, Tsuyuki, & Takahashi, 1973) (see Table 2) are given here for the first time, the assignments being made by comparison with those of friedelin.

Caryophyllene oxide (**7**) has been found in the essential oils of a number of plants belonging to a variety of families, e.g., Myrtaceae (Zhen, Kennery, & Lam, 1992), Dipterocarpaceae (Gupta, & Dev, 1971), Zingiberaceae (Damodaran, & Dev, 1968), Labiatae (Maurer, & Hauser, 1983). Recently, it was isolated



(4) R = CH$_2$
(6) R = α-CH$_3$,β-OH



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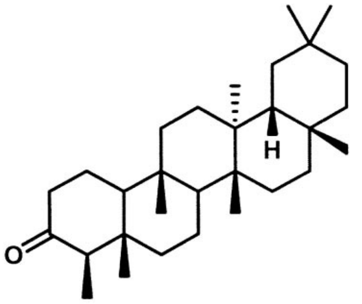

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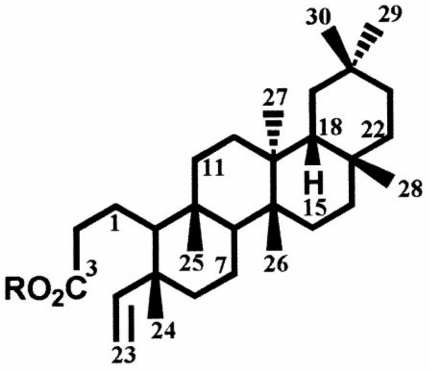


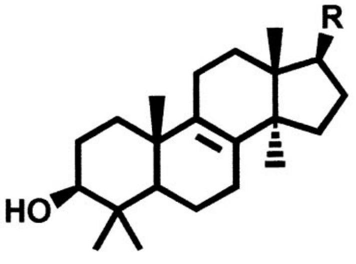
(9) R = H
(10) R = CH$_3$

Table 3
^{13}C NMR Shifts for Compounds **2**, **11**, **14** [41], **15** [18] and **17**.

Carbon	**2**[a]	**11**[a]	**14**[b]	**15**[c]	**17**[a]
1	35.3	35.3	35.6	31.7	35.6
2	28.0	27.9	27.8	26.9	27.9
3	79.0	79.0	79.0	80.8	79.0
4	38.9	38.9	38.9	39.5	38.9
5	51.0	51.0	50.4	47.3	50.4
6	19.0	18.9	18.2	20.9	18.3
7	27.7	27.7	26.5	28.1	26.5
8	134.0	134.0	134.4	47.8	134.5
9	133.6	133.5	134.4	20.2	134.4
10	37.3	37.3	37.0	26.1	37.1
11	21.5	21.5	21.0	25.8	21.0
12	30.9	31.0	30.9	35.6	30.93
13	44.1	44.2	44.4	45.4	44.5
14	50.0	50.0	49.8	48.9	49.9
15	29.8	29.8	30.8	32.9	30.89
16	28.2	27.9	28.2	26.6	28.2
17	49.7	49.6	50.4	52.1	50.2
18	15.6	15.8	15.7	18.0	15.8
19	20.2	20.2	19.1	29.7	19.2
20	35.9	36.2	36.2	36.5	36.7
21	18.9	19.1	18.6	18.3	18.7
22	35.4	38.2	36.3	39.1	39.1
23	24.8	125.7	24.9	125.7	125.6
24	125.2	139.3	125.2	139.5	139.4
25	130.8	70.8	130.9	70.7	70.8
26	17.7	29.88	25.7	29.96	29.9
27	25.8	29.93	17.6	30.05	30.0
28	28.1	28.1	27.9	19.3	28.0
29	15.5	15.5	15.4	15.2	15.4
30	24.5	24.5	24.2	25.5	24.3

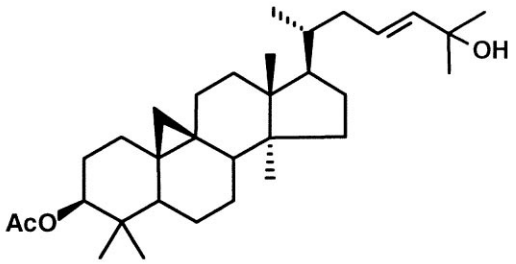
In $CDCl_3$. [a] 125 MHz, [b] 75 MHz, [c] 50.3 MHz.

from the roots and rhizomes of *Valeriana fauriei* (Valerianaceae) (Nishiya, Kimura, Takeya, & Itokawa, 1992) and the pods of *Sindora sumatrana* (Leguminosae) (Heymann 1994). Putranjivic acid (**9**) and its methyl ester (**10**) were first isolated from the leaves of *Putranjiva roxburghii* (Euphorbiaceae) (Chopra, Jain & Seshadri, 1969).



(14) R = A
(17) R = B
(19) R = C
(20) R = D

In addition to the known compounds, the most polar compound from the leaves, (20*R*,23*E*)-eupha-8,23-diene-3β,25-diol (**11**), was obtained as a gum, $C_{30}H_{50}O_2$ (*m/z* 442.3806), $[\alpha]_D + 17.0$. Its IR spectrum showed a characteristic hydroxyl absorption band (3480 cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra (see Table 3) exhibited resonances for a disubstituted double bond [δ_H 5.58 (2H, *br s*, H-23 and H-24); δ_C 139.3 and 125.7 (each *d*, C-23 and C-24)], a fully substituted double bond [δ_C 134.0 and 133.5 (each *s*, C-8 and C-9)], a secondary alcohol methine [δ_H 3.23 (1H, *dd*, $J = 4.5$ and 11.7, H-3α; δ_C 79.0 (*d*, C-3)], a tertiary



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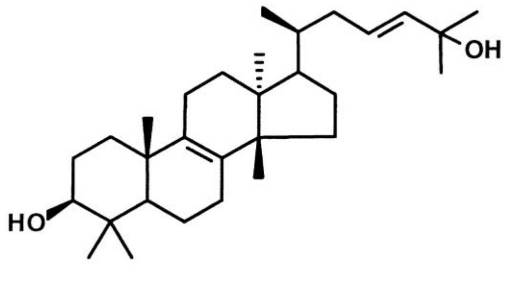

(16)

alcohol carbon [δ_C 70.8 (*s*, C-25)], one secondary [δ_H 0.82 (3H, *d*, $J = 6.0$ Hz, H_3-21)] and seven tertiary methyl groups [δ_H 1.31 (6H, *s*, H_3-26 and H_3-27), 1.00, 0.95, 0.88, 0.80 and 0.78 (each 3H, *s*, CH_3)], in addition to nine methylene carbons, three methine carbons and four quaternary carbons. The molecular formula indicated the presence of six units of unsaturation and since there are only two olefinic groups, the compound must be tetracarbocyclic.

The compound formed a monoacetate (**12**) [ν_{max} 1733 cm^{-1} (C=O), 1246 and 1026 cm^{-1} (C–O); δ_H 2.05 (3H, *s*, CH_3CO)] upon treatment with acetic anhydride/pyridine and a ketone **13** [ν_{max} 1708 cm^{-1} (C=O)] upon oxidation with pyridinium chlorochromate. The similarity between the A ring carbon shifts of **11** and those of euphol (**2**) and lanosterol (**14**) (see Table 3) indicated that C-3 is β-hydroxylated.

Comparison of the ^{13}C NMR shifts of the ring carbons of compound **10** with those of lanosterol (**14**) (see Table 3) revealed differences in the chemical shifts of C-6, C-7, C-9, C-15, C-17 and C-19 ($\Delta\delta_C$ 0.7, 1.2, 0.9, 1.0, 0.8 and 1.1). On the other hand, the nuclear carbon shifts for compounds **2** and **11** were virtually identical. This suggested that **11** had a euphane/tirucallane nucleus with a $\Delta^{8,9}$ double bond. The other double bond was hence located in the side chain. The overlapping signals at δ_H 5.58, attributed to two olefinic protons, did not offer much information as to the nature of the double bond. However, when the ^1H NMR spectrum was recorded in C_6D_6, the signal changed to an AB quartet with one member further coupled to a neighbouring methylene [δ_H 5.67 (1H, *ddd*, $J = 5.9$, 7.4 and 15.6 Hz, H-23), and 5.61 (1H, *d*, $J = 15.6$ Hz, H-24)], establishing the presence of a *trans*-double bond in the side chain. The deshielded nature (δ_H 1.31) of the C-25 methyls (H_3-26 and H_3-27) indicated that they must be attached to the fully substituted oxygenated carbon (δ_C 70.8, C-25) which, in turn, must carry the tertiary hydroxyl group. The structure of the side chain is therefore [$-CH_2-CH=CH-C(CH_3)_2OH$] which is a fairly common side chain in fungal lanostanes (Vrkoc, Budesinsky, & Dolejs, 1976) and is also known from higher plants (De Pascual Teresa, 1987). The ^{13}C NMR shifts of the side chain were virtually identical to the shifts of the corresponding carbons of (23*E*)-3β-acetoxycycloart-23-en-25-ol (**15**) (see Table 3) from *Euphorbia broteri* (De Pascual Teresa et al., 1987). The compound was therefore (20*R*,23*E*)-eupha-8,23-diene-3β,25-diol (**11**) or its 20-epimer (20*S*,23*E*)-tirucalla-8,23-diene-3β,25-diol (**16**).

In order to determine the C-20 configuration, **11** was prepared by photo-oxygenation of euphol (**2**). This reaction is well-known and has been previously carried out for the preparation of (23*E*)-lanosta-8,23-diene-3β,25-diol (**17**) from lanosterol acetate (Nagano, Poyser, Cheng, Luu et al., 1977). The physical properties of synthetic **11** were identical to those of the natural product and established its identity as (20*R*,23*E*)-eupha-8,23-diene-3β,25-diol. The other product of the photo-oxygenation was one of the two 24-hydroxy isomers of eupha-8,25-diene-3β,24ξ-diol (**18**). The configuration at C-24, however, was not assigned. The ^{13}C assignments for the nuclei of compounds **11** and **18** were made by comparison with those of euphol (**2**) while the shifts for the side chains of both **11** and **18** were assigned by comparison with the corresponding carbons of compound **15** (see Table 3).

The photo-oxygenation of lanosterol (**13**) was repeated since the ^{13}C NMR shifts for (23*E*)-lanosta-8,23-diene-3β,25-diol (**17**) were not available in the literature and they are useful for purposes of comparison. In addition to **17**, an inseparable mixture of the 24*R*- and 24*S*-isomers of **19** was also obtained. Oxidation of this mixture with MnO_2 gave 3β-hydroxylanosta-8,25-dien-24-one (**20**). The ^{13}C NMR shifts of **17** and **20** are reported below (see Experimental Section 3).

3. Experimental

Mps: uncorr. $[\alpha]_D$: $CHCl_3$. UV: EtOH. IR: CCl_4. NMR: 500 MHz (^1H) and 125 MHz (^{13}C) in $CDCl_3$ relative to TMS at $\delta = 0.00$. ^{13}C multiplicities were determined using the DEPT pulse sequence. CC: silica gel (Baker, 40 μm) or C_{18} (Bakerbond, 40 μm). GPC: Sephadex LH-20 ($CHCl_3$–MeOH, 1:1). HPLC: silica gel (Partisil, 5 μm, 4.6 × 250 mm) or C_{18} (Whatman ODS2, 5 μm, 4.6 × 250 mm); RI detection.

The leaves and bark of *Tripetalum cymosum* were obtained from the Forest Research Institute, Lae, Papua New Guinea (voucher no. 8866). A specimen (TC1) is retained in the Dept. of Chemistry, NUS. The samples were air dried and ground. The ground materials (1 kg bark, 280 g leaves) were then subjected to exhaustive extraction using hot hexane and concentrated in vacuo to afford the crude leaf extract (4.8 g) and the crude bark extract (1.8 g).

3.1. Bark extract

CC of the extract (silica gel, 3–5% EtOAc–hexane gradient) afforded two frs. HPLC [silica gel, 6.25% EtOAc–hexane followed by C_{18}, Me_2CO–H_2O–AcOH (88:11:1)] of fr 1 (708 mg) afforded glutin-5-en-3β-ol (**1**) (10.8 mg), euphol (**2**) (367 mg) and eupha-7,24-dien-3β-ol (butyrospermol) (**3**) (20 mg). Fr 2 (263 mg) was also subjected to HPLC [silica gel, 6.25% EtOAc–hexane, followed by C_{18}, Me_2CO–H_2O–AcOH (88:11:1)] to give **2** (147 mg), **3** (30 mg) and dammara-20,24-dien-3β-ol (**4**) (4.6 mg).

*3.2. Glutin-5-en-3β-ol (**1**)*

Colourless solid, mp. 207–208° (CH_3OH-$CHCl_3$) (lit. 209–210°C (Mahato, Das, & Sahu, 1981); $[\alpha]_D + 52.5$ (*c* 0.32) (lit. +61.0 [17]). FT–IR ν_{max} cm^{-1}: 3375 (OH), 1448 and 1373 (*gem*-dimethyl). EI–MS *m/z* (rel. int.): 426 $[M]^+$ (15), 408 $[M\text{-}H_2O]^+$ (23), 274 $[C_{20}H_{34}]^+$ (100), 259 $[C_{20}H_{34}\text{-}CH_3]^+$ (78), 205 (50), 152 $[C_{10}H_{16}O]^+$ (13), 137 (52), 134 $[C_{10}H_{14}]^+$ (82), 121 (51), 119 (49), 109 (53), 95 (63), 81 (53), 69 (62), 55 (74); HREI–MS: *m/z* 426.3859 ($C_{30}H_{50}O$ requires *m/z* 426.3862). ^1H NMR and ^{13}C NMR spectra were identical to those in the literature (Kitajima, Arai, & Tanaka, 1994; Carvalho, & Seita, 1993).

*3.3. Eupha-8,24-dien-3β-ol (Euphol) (**2**)*

Colourless needles, mp. 114–115°C (CH_3OH) (lit. 115–116°C (De Pascual Teresa, 1987); $[\alpha]_D$ +30.0 (*c* 0.30), +29.4 (*c* 4.40, C_6H_6) (lit. +31.0, $CHCl_3$ [18]). FT–IR ν_{max} cm^{-1}: 3481 (OH), 1670, 1454 and 1374 (*gem*-dimethyl), 1024 (C–O). EI–MS *m/z* (rel. int.): 426 $[M]^+$ (21), 135 (15), 133 (15), 121 (20), 119 (16), 109 (37), 107 (20), 105 (18), 95 (28), 81 (27), 79 (12), 69 (100), 67 (18), 55 (46), 43 (29), 41 (59); HREI–MS: *m/z* 426.3860 ($C_{30}H_{50}O$ requires *m/z* 426.3862). ^1H NMR: see Table 9; ^{13}C NMR: see Table 3.

3.4. Euphol acetate

The alcohol **1** (10 mg) was acetylated with Ac_2O–pyridine. After the usual workup, CC of the residue (silica gel, 1% EtOAc–hexane) yielded the monoacetate (**5**) (9 mg) as a colourless solid, mp. 107–109° (CH_3OH) (lit. 107–109°C (De Pascual Teresa, 1987). $[\alpha]_D + 37.2$ (*c* 0.91) (lit. +38.5 [18]). IR ν_{max} cm^{-1}: 1724 (C=O), 1443 and 1361 (*gem*-dimethyl), 1242 and 1019 (C–O). EI–MS *m/z* (rel. int.): 468 $[M]^+$ (18), 453 $[M\text{-}CH_3]^+$ (57), 408 $[M\text{–}AcOH]^+$ (9), 393 $[M\text{–}CH_3\text{–}AcOH]^+$ (58), 109 (41), 95 (36), 69 (100), 55 (49). HREI–MS: *m/z* 468.3939 ($C_{32}H_{52}O_2$ requires *m/z* 468.3967); ^1H NMR (300 MHz): δ 5.10 (1H, *br t*, *J* = 7.0 Hz, H-24), 4.50 (1H, *dd*, *J* = 4.7 and 11.7 Hz, H-3α), 2.05 (3H, *s*, CH_3CO), 1.69, 1.61 (each 3H, *s*, H_3-26, H_3-27), 0.98, 0.884 (each 3H, *s*, CH_3), 0.876 (6H, *s*, $2 \times CH_3$), 0.86 (3H, *d*, *J* = 6.2 Hz, H_3-21), 0.75 (3H, *s*, CH_3); ^{13}C NMR (300 MHz): δ 171.0 (*s*), 134.0 (*s*), 133.7 (*s*), 130.9 (*s*), 125.2 (*d*), 81.0 (*d*), 51.1 (*d*), 50.1 (*s*), 49.7 (*d*), 44.2 (*s*), 37.9 (*s*), 37.2 (*s*), 35.9 (*d*), 35.4 (*t*), 35.0 (*t*), 30.9 (*t*), 29.8 (*t*), 28.1 (*t*), 28.0 (*q*), 27.6 (*t*), 25.7 (*q*), 24.8 (*t*), 24.5 (*q*), 24.3 (*t*), 21.6 (*t*), 21.3 (*q*), 20.2 (*q*), 18.92 (*q*), 18.86 (*t*), 17.7 (*q*), 16.6 (*q*), 15.6 (*q*).

*3.5. Eupha-7,24-dien-3β-ol (Butyrospermol) (**3**)*

Gum (lit. 108–110°C (De Pascual Teresa, et al., 1987); $[\alpha]_D - 4.5$ (*c* 0.70) (lit. −12.0 [18]). FT–IR ν_{max} cm^{-1}: 3448 (OH), 1458 and 1376 [*gem*-dimethyl]. EI–MS *m/z* (rel. int.): 426 $[M]^+$ (26), 411 $[M\text{-}CH_3]^+$ (100), 393 (48). HREI–MS: *m/z* 426.3835 ($C_{30}H_{50}O$ requires *m/z* 426.3862); ^1H and ^{13}C NMR spectra identical to lit. values (De Pascual Teresa, 1987).

*3.6. Dammara-20,24-dien-3β-ol (**4**)*

Colourless solid, mp. 133–134°C (CH_3OH) (lit. 136–138°C (Mills, & Werner, 1955); $[\alpha]_D$ + 28.5 (*c* 0.40) (lit. +47 [20]). FT–IR ν_{max} cm^{-1}: 3500 (OH), 1637 (C=C), 1452 and 1376 (*gem*-dimethyl). EI–MS *m/z* (rel. int.): 426 $[M]^+$ (18), 357 (5); HREI–MS: *m/z* 426.3851 ($C_{30}H_{50}O$ requires *m/z* 426.3862). ^1H NMR: δ 5.13 (1H, *t sept*, *J* = 1.4 and 7.0 Hz, H-24), 4.74 (1H, *br s*, H-21), 4.71 (1H, *br s*, *J* = 1.5 Hz, H-21), 3.20 (1H, *dd*, *J* = 4.9 and 11.3 Hz, H-3α), 1.69, 1.62 (each 3H, *br s*, H_3-26 and H_3-27), 0.980, 0.977, 0.87, 0.85, 0.78 (each 3H, *s*, CH_3), 0.74 (1H, *dd*, *J* = 2.3 and 12.0 Hz, H-5). ^{13}C NMR: see Table 1. Difference NOE: H_3-28 [H-3α (4.8), H-5], H_3-30 [H_3-19 (0.8)], H_3-19 [H_3-30 (2.2), H_3-29 (1.1)], H_3-29 [H_3-28 (0.6), H_3-19 (1.7)].

3.7. Leaf extract

CC on silica gel (acetone–hexane gradient) gave sixteen frs. GPC of fr 1 (398 mg) followed by recrystallisation ($CHCl_3$–hexane) gave friedelin (**8**) (22 mg) which was identified by comparison with an authentic sample. CC (silica gel, 4% EtOAc–hexane) and HPLC (silica gel, 2% EtOAc–hexane) of the concentrated mother liquors (114 mg) afforded caryophyllene oxide (**7**) (10.5 mg). Fr 2 (735 mg) was subjected to GPC, CC (silica gel, 7% acetone–hexane) and HPLC (silica gel, 1% acetone–hexane) to give euphol (**2**) (323 mg). Fr 3 contained mainly **2** and was not further investigated. Fr 4 contained mainly putranjivic acid (**9**). Fr 5 (242 mg) was subjected to GPC and CC (C_{18}, 85% acetone–H_2O), followed by HPLC (C_{18}, 90% acetone–H_2O) to afford putranjivic acid (**9**) (30 mg). Fr 6 (293 mg) was not investigated as TLC of this fraction revealed no distinct spots. Frs 7 and 8 (534 mg) were combined, and upon GPC, CC (twice, C_{18}, 76% acetone–water, followed by silica gel, CH_2Cl_2) and final purification by HPLC (silica gel, 12% acetone–hexane) afforded (20*R*,23*E*)-eupha-8,23-diene-3β,25-diol (**11**) (18 mg).

*3.8. (6R,7R)-Caryophyllene oxide (**7**)*

Colourless solid, mp. 57–58° (CH_3OH) (lit. 61–62°C (Nishiya et al., 1992); $[\alpha]_D - 63.1$ (*c* 0.70) (lit. −66.7 (Nishiya et al., 1992). FT–IR ν_{max} cm^{-1}: 2932, 1630, 1454, 1383, 864. HREI–MS: *m*/*z* 220.1834 ($C_{15}H_{24}O$ requires *m*/*z* 220.1827); ^{1}H and ^{13}C NMR spectra identical to literature values (Heymann et al., 1994).

*3.9. Putranjivic acid (**9**)*

Colourless needles, mp. 178–180°C (CH_3OH) (lit. 173.0–173.5°C (Aoyagi, Tsuyuki, & Takahashi, 1973); $[\alpha]_D - 4.6$ (*c* 0.51) (lit. −12 (Aoyagi, Tsuyuki, & Takahashi, 1973). FT–IR ν_{max} cm^{-1}: 3283–2614 (carboxylic OH), 1708 (C=O). EI–MS *m*/*z* (rel. int.): 442 $[M]^+$ (6), 427 (10), 205 (66); HREI–MS: *m*/*z* 442.3814 ($C_{30}H_{50}O_2$ requires *m*/*z* 442.3811). ^{1}H NMR: δ 5.62 (1H, *dd*, *J* = 10.7 and 17.4 Hz, H-4), 4.93 (1H, *d*, *J* = 10.7 Hz, H-23*E*), 4.91 (1H, *d*, *J* = 17.4 Hz, H-23*Z*), 2.33 (2H, *m*, H_2-2), 1.18 (3H, *s*, H_3-5), 1.02 (3H, *s*, CH_3), 1.00 (6H, *s*, $2 \times CH_3$), 0.95 (3H, *s*, CH_3), 0.89 (3H, *s*, CH_3). ^{13}C NMR: see Table 2.

3.10. Methylation of **9**

The acid (9 mg) was methylated with CH_2N_2. CC of the residue (silica gel, 1% EtOAc–hexane) afforded methyl putranjivate (**10**) (6.0 mg) as a colourless solid, mp. 135–137° (CH_3OH) (lit. 136.0–136.5°C (Aoyagi, Tsuyuki, & Takahashi, 1973); $[\alpha]_D - 5.5$ (*c* 0.58) (lit. −8.3 (Aoyagi, Tsuyuki, & Takahashi, 1973). FT–IR ν_{max} cm^{-1}: 1740 (C=O), 1241 and 1049 (C–O). EI–MS *m*/*z* (rel. int.): 456 $[M]^+$ (9), 441 $[M\text{-}CH_3]^+$ (14), 331 (8), 301 (9), 273 (23), 250 (19), 223 (52), 218 (57), 205 (85), 95 (100). HREI–MS: *m*/*z* 456.3988 ($C_{31}H_{52}O_2$ requires *m*/*z* 456.3967). ^{1}H NMR: δ 5.61 (1H, *d*, *J* = 10.8 and 17.4 Hz, H-4), 4.92 (1H, *d*, *J* = 10.7 Hz, H-23*E*), 4.90 (1H, *d*, *J* = 17.4 Hz, H-23*Z*), 3.63 (3H, *s*, OCH_3), 2.29 (2H, *m*, H_2-2), 1.17 (3H, *s*, H_3-5), 1.02 (3H, *s*, CH_3), 0.99 (6H, *s*, $2 \times CH_3$), 0.98 (3H, *s*, CH_3), 0.94 (3H, *s*, CH_3), 0.88 (3H, *s*, CH_3). ^{13}C NMR: see Table 2.

*3.11. (20R,23E)-Eupha-8,23-diene-3β,25-diol (**11**)*

Gum. $[\alpha]_D + 17.0$ (*c* 0.45). IR ν_{max} cm^{-1}: 3380 (OH), 1455 and 1374 (*gem*-dimethyl), 1025 (C–O). EI–MS *m*/*z* (rel. int.): 442 $[M]^+$ (4), 424 $[M\text{-}H_2O]^+$ (32), 409 $[M\text{–}H_2O\text{–}CH_3]^+$ (82), 391 $[M\text{–}2H_2O\text{–}CH_3]^+$ (42), 109 (100); HREI–MS: *m*/*z* 442.3806 ($C_{30}H_{50}O_2$ requires *m*/*z* 442.3811). ^{1}H NMR: δ 5.58 (2H, *br s*, H-23 and H-24), 3.23 (1H, *dd*, *J* = 4.5 and 11.7 Hz, H-3α), 2.34 (1H, *br d*, *J* = 12.7 Hz), 1.31 (6H, *s*, H_3-26 and H_3-27), 1.00, 0.95, 0.88 (each 3H, *s*, CH_3), 0.82 (3H, *d*, *J* = 6.0 Hz, H_3-21), 0.80, 0.78 (each 3H, *s*, CH_3). ^{1}H NMR (C_6D_6): δ 5.67 (1H, *ddd*, *J* = 5.9, 7.4 and 15.6 Hz, H-23), 5.61 (1H, *d*, *J* = 15.6 Hz, H-24), 3.07 (1H, *m*, H-3α), 2.43 (1H, *br d*, *J* = 8.1 Hz), 1.25 (6H, *s*, H_3-26 and H_3-27), 1.04, 0.96, 0.93 (each 3H, *s*, CH_3), 0.93 (3H, *d*, *J* = 6.1 Hz, H_3-21), 0.87, 0.85 (each 3H, *s*, CH_3). ^{13}C NMR: see Table 3.

3.12. Acetylation of **11**

The diol (**11**) (7.5 mg) was acetylated with Ac_2O–pyridine. The crude product was purified by HPLC (silica gel, 7% acetone–hexane) to give the monoacetate (**12**) (4 mg) as a colou‘rless solid, mp. 112–113° ($CHCl_3$). IR ν_{max} cm^{-1}: 1733 (ester C=O), 1456 and 1372 (*gem*-dimethyl), 1246 and 1026 (C–O). EI–MS *m*/*z* (rel. int.): 484 $[M]^+$ (25), 466 (23), 452 (84), 370 (89); HREI–MS: *m*/*z* 484.3930 ($C_{32}H_{52}O_3$ requires *m*/*z* 484.3916). ^{1}H NMR: δ 5.58 (2H, *m*, H-23 and H-24), 4.50 (1H, *dd*, *J* = 4.6 and 11.8 Hz, H-3α), 2.34 (1H, *br d*, *J* = 12.6 Hz), 2.05 (3H, *s*, CH_3CO), 1.31 (6H, *s*, H_3-26 and H_3-27), 0.98, 0.884 (each 3H, *s*, CH_3), 0.876 (6H, *s*, $2 \times CH_3$), 0.82 (3H, *d*, *J* = 5.9 Hz, H_3-21), 0.77 (3H, *s*, CH_3).

3.13. Oxidation of **11**

The alcohol **11** (11.5 mg) was oxidised with PCC in CH_2Cl_2. The crude product was purified by flash chromatography (silica gel, 7% acetone–hexane) to give the ketone **13** (2 mg) as a colourless solid, mp. 132–133° ($CHCl_3$). IR ν_{max} cm^{-1}: 1708 (C=O), 1459 and 1376 (*gem*-dimethyl). EI–MS *m*/*z* (rel. int.): 440 $[M]^+$ (11), 407 (70), 325 (97); HREI–MS: *m*/*z* 440.3640 ($C_{30}H_{48}O_2$ requires *m*/*z* 440.3654). ^{1}H NMR: δ 5.59 (2H, *m*, H-23 and H-24), 2.56 (1H, *ddd*, *J* = 7.5, 10.0 and 15.9 Hz, H-2α), 2.47 (1H, *ddd*, *J* = 3.9, 7.7 and 15.9 Hz, H-2β), 2.35 (1H, *br d*, *J* = 12.4 Hz), 1.32 (6H, *s*, H_3-26 and H_3-27), 1.10, 1.062, 1.057, 0.90 (each 3H, *s*, CH_3), 0.83 (3H, *d*, *J* = 6.0 Hz, H_3-21), 0.78 (3H, *s*, CH_3).

*3.14. Photo-oxygenation of euphol (**2**)*

A mixture of euphol (260 mg) and methylene blue (1.5 mg) in EtoH (30 ml) was irradiated with a 500 W lamp. The reaction mixture was stirred rapidly to effect the introduction of oxygen and its temperature was maintained at 25°. Upon consumption of the euphol, excess $NaBH_4$ was added and the reaction mixture was stirred for 15 mins. Water was then added to decompose unreacted $NaBH_4$ and the EtOH was distilled off. The crude product was partitioned between CH_2Cl_2 and water. The organic layer was dried with Na_2SO_4, concentrated and flash chromatographed (silica gel, 8% acetone–hexane) to give (20*R*)-eupha-8,25-diene-3β,24ξ-diol (**18**) (17 mg) and (20*R*,23*E*)-eupha-8,23-diene-3β,25-diol (**11**) (31 mg), identical to the natural product.

*3.15. (20R)-Eupha-8,25-diene-3β,24ξ-diol (*18*)*

Gum. $[\alpha]_D + 19.1$ (*c* 1.70). IR ν_{max} cm^{-1}: 3389 (OH), 1465 and 1374 (*gem*-dimethyl), 1025 (C–O). EI–MS *m/z* (rel. int.): 442 $[M]^+$ (25), 427 (34), 409 (100), 391 (42), 175 (27), 173 (24), 149 (27), 133 (36); HREI–MS: *m/z* 442.3801 ($C_{30}H_{50}O_2$ requires *m/z* 442.3811). ^1H NMR: δ 4.92, 4.83 (each 1H, *br s*, H_2-26), 4.01 (1H, *t*, $J = 6.1$ Hz, H-24), 3.23 (1H, *dd*, $J = 4.5$ and 11.7 Hz, H-3α), 1.72 (3H, *br s*, H_3-27), 0.99, 0.94, 0.86 (each 3H, *s*, CH_3), 0.85 (3H, *d*, $J = 6.2$ Hz, H_3-21), 0.79, 0.75 (each 3H, *s*, CH_3); ^{13}C NMR: δ 147.6 (*s*), 134.0 (*s*), 133.5 (*s*), 111.1 (*t*), 79.0 (*d*), 76.6 (*d*), 51.0 (*d*), 50.0 (*s*), 49.7 (*d*), 44.1 (*s*), 38.9 (*s*), 37.3 (*s*), 36.0 (*d*), 35.3 (*t*), 31.6 (*t*), 31.1 (*t*), 30.9 (*t*), 29.8 (*t*), 28.0 (*q*), 28.0 (*t*), 27.9 (*t*), 27.7 (*t*), 24.5 (*q*), 21.5 (*t*), 20.1 (*q*), 19.0 (*q*), 18.9 (*t*), 17.4 (*q*), 15.7 (*q*), 15.5 (*q*).

*3.16. Photo-oxygenation of lanosterol (*13*)*

Commercially obtained lanosterol (250 mg) (which contained dihydrolanosterol as impurity) was used. The procedure and the workup are as described above. The crude product was flash chromatographed (silica gel, 15% EtOAc–hexane) to give unreacted dihydrolanosterol (44.1 mg), (23*E*)-lanosta-8,23-diene-3β,25-diol (**17**) (25.4 mg) and a mixture of the 24-epimers of lanosta-8,25-diene-3β,24-diol (**19**) (25.5 mg).

*3.17. (23E)-Lanosta-8,23-diene-3β,25-diol (*17*)*

Colourless plates, mp. 197–198°C ($CHCl_3$), (lit. 192.5–193.5°C (Nagano et al., 1977); $[\alpha]_D + 46.5$ (*c* 1.40). IR ν_{max} cm^{-1}: 3450 (OH), 1454 and 1372 (*gem*-dimethyl). EI–MS *m/z* (rel. int.): 442 $[M]^+$ (3), 424 $[M–H_2O]^+$ (12), 409 $[M–H_2O–CH_3]^+$ (35), 391 $[M–2H_2O–CH_3]^+$ (27), 109 (100); HREI–MS: *m/z* 442.3802 ($C_{30}H_{50}O_2$ requires *m/z* 442.3811). ^1H NMR: δ 5.59 (2H, *br s*, H-23 and H-24), 3.23 (*dd*, $J = 4.5$ and 11.6 Hz, H-3α), 1.31 (6H, *s*, H_3-26 and H_3-27), 1.00, 0.98 (each 3H, *s*, CH_3), 0.89 (3H, *d*, $J = 5.9$ Hz, H_3-21), 0.87, 0.81, 0.69 (each 3H, *s*, CH_3). ^{13}C NMR: see Table 3.

*3.18. Mixture of 24-epimers of lanosta-8,25-diene-3β,24-diol (*19*)*

^1H NMR: δ 4.92 (2H, quintet of *d*, $J = 0.9$, 5.7 Hz, $2 \times$ H-26), 4.83 (2H, *m*, $2 \times$ H-26), 4.01 (2H, *t*, $2 \times$ H-24), 3.23 (2H, *dd*, $J = 4.5$ and 11.6 Hz, $2 \times$ H-3α), 1.72 (6H, *br s*, $2 \times H_3$-27), 1.00, 0.98 (each 6H, *s*, $2 \times CH_3$), 0.91 (6H, *d*, $J = 6.3$ Hz, $2 \times H_3$-21), 0.87, 0.81, 0.69 (each 6H, *s*, $6 \times CH_3$). ^{13}C NMR: δ 147.8 (*s*), 147.5 (*s*), 134.5 (*s*), 134.4 (*s*), 111.4 (*t*), 110.9 (*t*), 79.0 (*d*), 76.8 (*d*), 76.3 (*d*), 50.4 (*d*), 50.3 (*d*), 49.8 (*s*), 44.5 (*s*), 38.9 (*s*), 37.1 (*s*), 36.3 (*d*), 35.6 (*t*), 31.95 (*t*), 31.92 (*s*), 31.7 (*t*), 31.5 (*t*), 31.0 (*t*), 30.9 (*t*), 28.2 (*t*), 28.1 (*t*), 28.0 (*q*), 27.9 (*t*), 26.5 (*t*), 24.3 (*q*), 21.0 (*t*), 19.2 (*q*), 18.7 (*q*), 18.3 (*t*), 17.6 (*q*), 17.2 (*q*), 15.8 (*q*), 15.4 (*q*).

*3.19. β-Hydroxylanosta-8,25-dien-24-one (*20*)*

The mixture of 24-epimers of **19** (8 mg) was dissolved in C_6H_6. Activated MnO_2 in C_6H_6 was added to the soln and the reaction mixture was stirred and monitored by TLC. MnO_2 was removed by filtration through Celite and the crude product obtained was purified by HPLC (DIOL, 10% EtOAc–hexane) to afford **20** (4.1 mg) as a colourless solid, mp. 152–153° ($CHCl_3$). IR ν_{max} cm^{-1}: 1682 (C=O), 1453 and 1372 (*gem*-dimethyl). EI–MS *m/z* (rel. int.): 440 $[M]^+$ (16), 425 (53), 407 (70); HREI–MS: *m/z* 440.3648 ($C_{30}H_{48}O_2$ requires *m/z* 440.3654). ^1H NMR: δ 5.95 (1H, *br s*, H-26), 5.75 (1H, *br s*, H-26), 3.23 (1H, *dd*, $J = 4.6$ and 11.6 Hz, H-3α), 2.72 (1H, *ddd*, $J = 5.3$, 10.3 and 15.8 Hz, H-23), 2.61 (1H, *ddd*, $J = 5.9$, 10.0 and 15.8 Hz, H-23), 1.87 (3H, *br s*, H_3-27), 0.91 (3H, *d*, $J = 6.3$ Hz, H_3-21), 1.00, 0.98, 0.88, 0.81 and 0.70 (each 3H, *s*, CH_3). ^{13}C NMR: 202.9 (*s*), 144.6 (*s*), 134.5 (*s*), 134.4 (*s*), 124.2 (*t*), 79.0 (*d*), 50.4 ($2 \times d$), 44.6 (*s*), 38.9 (*s*), 37.1 (*s*), 36.3 (*d*), 35.6 (*t*), 34.7 (*t*), 31.1 (*t*), 31.0 (*t*), 30.8 (*t*), 28.2 (*t*), 28.0 (*q*), 27.9 (*t*), 26.5 (*t*), 24.3 (*q*), 21.0 (*t*), 19.2 (*q*), 18.6 (*q*), 18.3 (*t*), 17.8 (*q*), 15.8 (*q*), 15.4 (*q*).

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