

## Proteasome Inhibitors from *Neoboutonia melleri*

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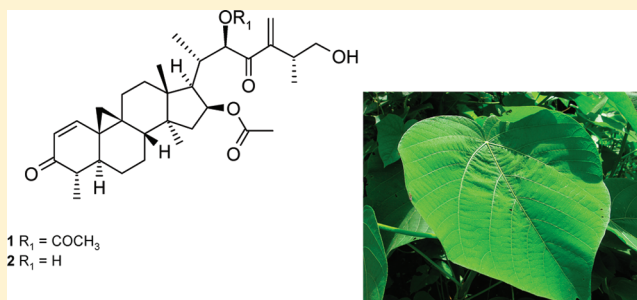
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### S Supporting Information

**ABSTRACT:** Thirty new cycloartane derivatives (1–3, 5–12, 14–32) have been isolated from the leaves of *Neoboutonia melleri*. Their novelty stems from the loss of one of the C-4 methyl groups (1–3, 5–12, 14–25, and 32) and from the presence of an “extra” carbon atom in the side chain (1–3, 5–12, 14–20, 26–29, and 30–32). Furthermore, compound 32 possesses a rare triterpene skeleton with the cyclopropane ring fused onto C-1 and C-10, instead of C-9 and C-10. The structures were determined by spectrometric means, chemical correlations, and X-ray crystallography of derivative 1c. The substitution pattern in ring A, with a cyclopropyl ring conjugated with an  $\alpha,\beta$ -unsaturated carbonyl moiety, confers to the molecule a particular reactivity, giving rise to a formal inversion of the stereochemistry of the cyclopropane ring under UV irradiation. These compounds showed an interesting level of activity on the proteasome pathway, thus motivating their evaluation as possible anticancer agents. The large number of isolated compounds permitted a structure–activity relationship analysis, which showed that the presence of the two enone functions was a requirement for the activity.



For the past decade, proteasome inhibitors have been considered as potentially useful drugs in the treatment of cancer, and this has culminated in the approval of bortezomib for the treatment of multiple myeloma.<sup>1</sup> Epoxomicin, lactacystin, and, the most promising, salinosporamide A, presently in phase 1 clinical trials, are examples of natural products that inhibit the proteasome catalytic functions.<sup>2</sup> In order to find new inhibitors that do not particularly target proteolysis but the whole ubiquitin–proteasome pathway, we have developed an assay based on a human colon cancer cell line that stably expresses a 4-ubiquitin luciferase reporter protein (4Ub-Luc DLD-1).<sup>3</sup> Under normal conditions, the fusion protein (4Ub-Luc) is produced and, due to the ubiquitin tag's presence, it is addressed to and degraded by the proteasome, while inhibitors of the proteasome pathway lead to protein accumulation and luminescence observation.<sup>4</sup>

The assay was applied to a collection of over 12 000 plant extracts and 62 000 pure compounds including natural products and derivatives. An extract from *Neoboutonia melleri* (Muell. Arg.) Prain and two pure compounds isolated from it (1 and 2) inhibited the proteasome pathway. The plant was collected in Cameroon, in the late 1980s, following ethnopharmacological observations. Its leaves were locally used to wrap fish, hence, its name

Koutencha, “fish leaves” in the Bamum language. *Neoboutonia* is an African tropical genus of the Euphorbiaceae, which, according to the latest classifications, represents numerous varieties combined into three species: *N. melleri*, *N. diaguissensis* Beille, and *N. macrocalyx* Pax.<sup>5</sup> *N. melleri*, a small tree (4–8 m) growing in swamp areas from Cameroon/Angola to Sudan/Mozambique, exhibits alternate, stipulate leaves, broadly ovate or subcircular. It is locally considered as toxic and of interest against diabetes. *N. melleri* was the subject of a single phytochemical investigation,<sup>6</sup> and articles on the genus are rare.<sup>7</sup> Here we describe the isolation and structural characterization of 30 new compounds, evaluation of their biological activity on the proteasome pathway, and discussion of their structure–activity relationship.

### RESULTS AND DISCUSSION

The CH<sub>2</sub>Cl<sub>2</sub> extract of *N. melleri* was dominated by two compounds, which differed by the presence of an acetyl group and

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Table 1.  $^{13}\text{C}$  NMR Data of Compounds 1-8

carbon	1 <sup>a</sup>	2 <sup>a</sup>	3 <sup>b</sup>	4 <sup>b</sup>	5 <sup>b</sup>	6 <sup>b</sup>	7 <sup>a</sup>	8 <sup>a</sup>
1	153.7, CH	153.9, CH	155.5, CH	155.3, CH	33.5, CH	33.5, CH	154.4, CH	154.6, CH
2	128.3, CH	128.2, CH	128.4, CH	128.5, CH	41.5, CH	41.5, CH	127.4, CH	127.3, CH
3	202.0, C	202.1, C	202.4, C	202.3, C	213.2, C	213.3, C	202.5, C	202.7, C
4	46.9, CH	46.9, CH	47.6, CH	47.5, CH	50.5, CH	50.5, CH	43.8, CH	43.8, CH
5	42.5, CH	42.6, CH	43.6, CH	43.1, CH	47.0, CH	47.0, CH	45.7, CH	45.7, CH
6	23.4, CH <sub>2</sub>	23.6, CH <sub>2</sub>	24.3, CH <sub>2</sub>	23.9, CH <sub>2</sub>	26.5, CH <sub>2</sub>	26.5, CH <sub>2</sub>	65.6, CH	65.6, CH
7	23.5, CH <sub>2</sub>	23.6, CH <sub>2</sub>	24.3, CH <sub>2</sub>	23.8, CH <sub>2</sub>	26.2, CH <sub>2</sub>	26.2, CH <sub>2</sub>	31.9, CH <sub>2</sub>	31.9, CH <sub>2</sub>
8	44.3, CH	44.7, CH	45.2, CH	43.6, CH	48.6, CH	48.6, CH	40.1, CH	40.2, CH
9	26.0, C	26.1, C	27.2, C	27.4, C	25.4, C	25.4, C	25.3, C	25.4, C
10	31.9, C	31.9, C	32.9, C	33.3, C	30.3, C	30.3, C	29.6, C	29.7, C
11	27.4, CH <sub>2</sub>	27.5, CH <sub>2</sub>	28.1, CH <sub>2</sub>	28.4, CH <sub>2</sub>	27.4, CH <sub>2</sub>	27.4, CH <sub>2</sub>	27.1, CH <sub>2</sub>	27.2, CH <sub>2</sub>
12	32.1, CH <sub>2</sub>	32.1, CH <sub>2</sub>	32.9, CH <sub>2</sub>	32.8, CH <sub>2</sub>	33.3, CH <sub>2</sub>	33.5, CH <sub>2</sub>	32.3, CH <sub>2</sub>	32.3, CH <sub>2</sub>
13	47.4, C	47.4, C	46.9, C	44.8, C	46.8, C	46.7, C	46.3, C	46.1, C
14	46.0, C	45.8, C	48.4, C	52.4, C	48.0, C	47.9, C	46.7, C	46.7, C
15	46.0, CH <sub>2</sub>	46.2, CH <sub>2</sub>	46.7, CH <sub>2</sub>	41.7, CH <sub>2</sub>	47.6, CH <sub>2</sub>	47.8, CH <sub>2</sub>	46.8, CH <sub>2</sub>	46.9, CH <sub>2</sub>
16	76.0, CH	76.9, CH	76.7, CH	82.5, CH	76.9, CH	77.5, CH	76.2, CH	77.1, CH
16a	170.1, C	170.1, C	171.3, C		171.3, C	171.3, C	170.1, C	170.2, C
16b	21.7, CH <sub>3</sub>	21.8, CH <sub>3</sub>	22.1, CH <sub>3</sub>		22.1, CH <sub>3</sub>	22.1, CH <sub>3</sub>	21.7, CH <sub>3</sub>	21.8, CH <sub>3</sub>
17	50.3, CH	50.1, CH	51.3, CH	60.4, CH	51.4, CH	51.5, CH	50.5, CH	50.4, CH
18	17.8, CH <sub>3</sub>	18.2, CH <sub>3</sub>	18.3, CH <sub>3</sub>	21.3, CH <sub>3</sub>	19.3, CH <sub>3</sub>	19.5, CH <sub>3</sub>	19.0, CH <sub>3</sub>	19.3, CH <sub>3</sub>
19	27.0, CH <sub>2</sub>	27.4, CH <sub>2</sub>	27.6, CH <sub>2</sub>	26.5, CH <sub>2</sub>	28.0, CH <sub>2</sub>	28.0, CH <sub>2</sub>	32.6, CH <sub>2</sub>	32.7, CH <sub>2</sub>
20	32.4, CH	35.9, CH	33.2, CH	38.2, CH	33.2, CH	36.3, CH	32.4, CH	35.8, CH
21	12.8, CH <sub>3</sub>	11.7, CH <sub>3</sub>	13.3, CH <sub>3</sub>	15.8, CH <sub>3</sub>	13.2, CH <sub>3</sub>	12.2, CH <sub>3</sub>	12.7, CH <sub>3</sub>	11.5, CH <sub>3</sub>
22	77.6, CH	74.8, CH	78.3, CH	111.4, C	78.5, CH	75.8, CH	77.6, CH	74.8, CH
22a	170.6, C		171.6, C		171.7, C		170.6, C	
22b	20.7, CH <sub>3</sub>		20.9, CH <sub>3</sub>		20.9, CH <sub>3</sub>		20.7, CH <sub>3</sub>	
23	198.7, C	204.5, C	198.9, C	77.8, CH	199.6, C	205.6, C	198.6, C	204.4, C
24	149.3, C	148.0, C	149.2, C	153.3, C	150.3, C	149.1, C	149.2, C	147.9, C
24a	123.7, CH <sub>2</sub>	125.7, CH <sub>2</sub>	125.6, CH <sub>2</sub>	114.2, CH <sub>2</sub>	124.6, CH <sub>2</sub>	126.5, CH <sub>2</sub>	123.8, CH <sub>2</sub>	125.8, CH <sub>2</sub>
25	37.2, CH	36.7, CH	34.9, CH	38.3, CH	37.9, CH	37.6, CH	37.1, CH	36.6, CH
26	66.9, CH <sub>2</sub>	67.0, CH <sub>2</sub>	67.8, CH <sub>2</sub>	69.0, CH <sub>2</sub>	66.4, CH <sub>2</sub>	66.7, CH <sub>2</sub>	66.9, CH <sub>2</sub>	67.0, CH <sub>2</sub>
26a			171.6, C					
26b			21.1, CH <sub>3</sub>					
27	16.3, CH <sub>3</sub>	16.5, CH <sub>3</sub>	17.3, CH <sub>3</sub>	17.8, CH <sub>3</sub>	17.2, CH <sub>3</sub>	17.2, CH <sub>3</sub>	16.3, CH <sub>3</sub>	16.5, CH <sub>3</sub>
28	10.8, CH <sub>3</sub>	10.8, CH <sub>3</sub>	11.3, CH <sub>3</sub>	11.3, CH <sub>3</sub>	11.2, CH <sub>3</sub>	11.2, CH <sub>3</sub>	10.8, CH <sub>3</sub>	10.8, CH <sub>3</sub>
30	19.6, CH <sub>3</sub>	19.6, CH <sub>3</sub>	20.0, CH <sub>3</sub>	19.3, CH <sub>3</sub>	20.6, CH <sub>3</sub>	20.7, CH <sub>3</sub>	20.3, CH <sub>3</sub>	20.3, CH <sub>3</sub>

<sup>a</sup>In CDCl<sub>3</sub>. <sup>b</sup>In CD<sub>3</sub>CN.

were named neoboutomellerone (1) and 22-de-O-acetylneoboutomellerone (2). Compound 1 showed a pseudomolecular ion peak at  $m/z$  591.3  $[\text{M} + \text{Na}]^+$  in the positive ESI mode that analyzed for C<sub>34</sub>H<sub>48</sub>O<sub>7</sub>Na. The UV spectrum exhibited two maxima at 228 and 266 nm; the IR spectrum displayed strong OH vibrations at 3430 cm<sup>-1</sup> and carbonyl bands at 1735, 1665, and 1602 cm<sup>-1</sup>. Analysis of the  $^{13}\text{C}$  NMR spectrum (DEPTQ and HSQC) permitted identification of seven methyl, eight methylene, ten methine, and nine quaternary carbons, thus accounting for a C<sub>34</sub>H<sub>47</sub> partial formula and, therefore, a single OH group. Among the deshielded signals, two were clearly assignable to ketocarboxyls ( $\delta_{\text{C}}$  198.7 and 202.0), two ester carbonyls ( $\delta_{\text{C}}$  170.1 and 170.6), and an oxymethylene carbon at  $\delta_{\text{C}}$  66.9. The esters were identified as acetates on the basis of HMBC correlations between the CO and methyls at  $\delta_{\text{H}}$  2.08 and 2.16. The composition determined by HRMS was thus fully explained by NMR. A cyclohexenone moiety with a quaternary carbon atom  $\gamma$  to the CO, a conjugated *exo*-methylene, two acetates, two methyl groups on sp<sup>3</sup> quaternary carbons, and three methyl doublets were particular features of the molecule. The observation of an isolated methylene with its most upfield proton at  $\delta_{\text{H}}$  0.58, showing a small geminal coupling constant ( $J = 4.3$  Hz), suggested the presence of a cyclopropane

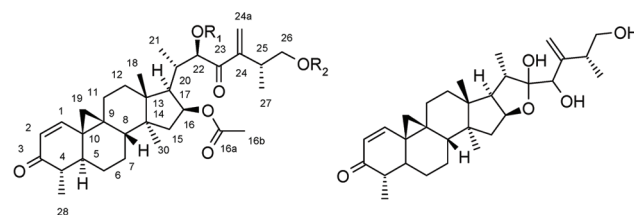
ring. Given the C<sub>30</sub> composition of the basic skeleton (C<sub>34</sub> minus two acetyl moieties), the first structural hypothesis for 1 was that of a diacetylated cycloartane derivative. On this basis, the two methyl singlets were assigned to C-18 and C-30, and the HMBC experiment permitted detection of the two quaternary carbons C-13 and C-14 (shared correlations with the methyls, see Table 1 for chemical shifts) as well as C-8, C-17 (CH), C-12, and C-15 (CH<sub>2</sub>). The cyclohexenone moiety was located in ring A to account for the observed long-range couplings between the ketocarboxyl (C-3), H-1, H-4, H-5, and CH<sub>3</sub>-28; the most upfield cyclopropane proton showed a correlation with H-1. Contrary to most described cycloartanes,<sup>8</sup> 1 had a proton on C-4 and therefore a single methyl group at this position. Analysis of the HMBC and HSQC experiments permitted identification of all the carbon atoms. The acetates were located on C-16 and C-22, and the alcohol function was located on the methyl group that terminates the chain. The location of the second enone moiety and the 30th carbon atom was determined by the couplings observed between the carbonyl at  $\delta_{\text{C}}$  198.7, the *exo*-methylene, and H-22, on one hand, and between the *exo*-methylene protons and C-26 (or -27), on the other. The extra carbon atom was thus placed at C-24, a common alkylation site in the cycloartane series.<sup>9</sup> The planar

structure of neoboutomellerone (**1**) is thus 16,22-di-*O*-acetyl-26-hydroxy-29-nor-24-methylcycloarta-1,24(24a)-diene-3,23-dione.

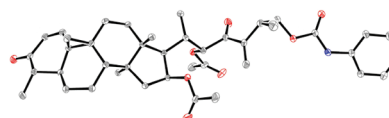
The second most abundant molecule was straightforwardly identified as 22-de-*O*-acetylneoboutomellerone (**2**), H-22 appearing shielded at  $\delta_{\text{H}}$  4.74 instead of 5.57. Upon acetylation, compounds **1** and **2** yielded the same 26-acetylneoboutomellerone (**3**), also isolated as a minor natural product, thus demonstrating that the three compounds had the same configuration. The latter was established through a ROESY experiment and by the preparation of Mosher esters. The cyclopropyl hydrogen atoms (H-19) were distinguished by the observation that the *exo* hydrogen showed ROESY correlations with H-1 and H-11*eq*, and the *endo* hydrogen with H-4, H-6, and H-8. These observations showed that the A/B ring junction was *trans* (H-5 $\alpha$ ) and that the C-4 methyl was equatorial (H-4 $\beta$ ). In ring D correlations between the angular methyl-18, H-8, and H-20 on one hand and between CH<sub>3</sub>-30, H-16, and H-17, on the other, permitted assignment of a C/D *trans* ring junction with OAc-16 and the side chain in a  $\beta$ -orientation. The configuration of C-25 was determined by examination of the Mosher esters of **1** (**1a** and **1b**), according to the method of Ivanchina.<sup>10</sup> Ester **1a**, prepared with *S*- $\alpha$ -methoxy- $\alpha$ -trifluoromethylphenylacetic acid chloride, showed 0.12 ppm nonequivalence between the diastereotopic H-26, while this value was 0.04 ppm for ester **1b** prepared with the *R*-acid chloride, thus implying a 25*R* configuration. The configuration of C-22 was established similarly on diol **2** by considering the chemical shifts of H-20; in this case H-20 in the ester **2a**, prepared from the *S*-acid chloride, was found at higher field than in the ester **2b** made with the *R*-reagent ( $\delta_{\text{H}}$  2.57 vs 2.64). Finally, the configuration of C-20 was determined by the examination of the NMR spectra of compound **4**, a saponification product of **1**, in which a *cis*-fused five-membered ring is formed following hydrolysis of the acetate at C-16,  $\alpha$ -ketol rearrangement, and ring closure as a hemiketal. In this compound, NOE correlations are observed between H-20 and CH<sub>3</sub>-18, between H-17 and CH<sub>3</sub>-21, and between CH<sub>3</sub>-30, H-16, and H-17, thus establishing a 20*S* configuration. Compounds **1** and **2** could not be crystallized for X-ray analysis. However, phenylcarbamate **1c** was obtained in crystalline form in the course of a chemical modification program. The crystal structure determination confirmed these assignments as depicted in the formulas (see Experimental Section and Supporting Information for details).

These molecules show a high degree of novelty, and their highly functionalized side chain is unique. Furthermore, there are only a few examples of nor-29-cycloarta-1-en-3-ones.<sup>11</sup> With these two major compounds and a valuable biological activity at hand, it was decided to enlarge the panel of molecules in order to evaluate the structural requirements for activity. A derivatization program was thus undertaken,<sup>12</sup> as well as a systematic search for related natural products contained in this particular plant and in a few plants well known as sources of cycloartanes (*Cimicifuga simplex*, *Musa sapientum*, and *Mangifera indica*).

Two minor compounds, **5** and **6**, respectively the 1,2-dihydro derivatives of **1** and **2**, were first isolated. Their HR-MS compositions were in agreement with the expected formulas (C<sub>34</sub>H<sub>50</sub>O<sub>7</sub> and C<sub>32</sub>H<sub>48</sub>O<sub>6</sub>), and their <sup>1</sup>H NMR spectra did not exhibit a deshielded AX system for H-1 and H-2. Both molecules showed a strong shielding of C-28 ( $\delta_{\text{C}}$  11.2), as a result of its equatorial position,  $\beta$  to a carbonyl group. All other <sup>13</sup>C NMR signals except those of rings A and B were similar to those of **1**–**3** (see Table 1). On the basis of these data, the structures of **5** and **6**



- 1** R<sub>1</sub> = COCH<sub>3</sub>, R<sub>2</sub> = H  
**2** R<sub>1</sub> = R<sub>2</sub> = H  
**3** R<sub>1</sub> = R<sub>2</sub> = COCH<sub>3</sub>  
**1a** R<sub>1</sub> = COCH<sub>3</sub>, R<sub>2</sub> = (*R*)-MTPA  
**1b** R<sub>1</sub> = COCH<sub>3</sub>, R<sub>2</sub> = (*S*)-MTPA  
**2a** R<sub>1</sub> = H, R<sub>2</sub> = (*R*)-MTPA  
**2b** R<sub>1</sub> = H, R<sub>2</sub> = (*S*)-MTPA  
**1c** R<sub>1</sub> = COCH<sub>3</sub>, R<sub>2</sub> = CONHPh



ORTEP representation of **1c**

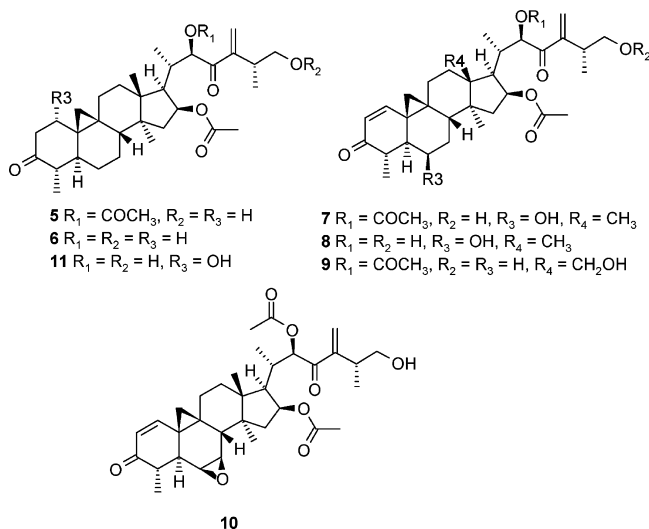
were identified as 1,2-dihydroneoboutomellerone and 1,2-dihydro-22-de-*O*-acetylneoboutomellerone.

HR-MS and NMR demonstrated that the pair of new compounds **7** and **8** contained an extra oxygen atom compared to their respective parent compounds **1** and **2**. The <sup>1</sup>H NMR spectra showed a broad singlet ( $W_{1/2}$  = 10 Hz) at  $\delta_{\text{H}}$  4.20 in the new compounds, corresponding to a CH at  $\delta_{\text{C}}$  65.6. In the COSY experiment, this signal displayed couplings to upfield CH and CH<sub>2</sub>, but the HMBC failed to yield any useful correlations. Assuming that the basic skeleton of the neoboutomellerones was conserved, C-6 was left as the unique position for the hydroxylation. In agreement, the neighboring C-5 and C-7 were downfield shifted (4 and 9 ppm, respectively), while C-8 exhibited a  $\gamma$  shielding effect (3 ppm). This substitution also induced a strong deshielding of C-19 ( $\Delta\delta$  +6,  $\delta$  effect) and, most importantly, of the *endo* proton of the cyclopropane ( $\Delta\delta_{\text{H}}$  +0.63) moiety, which could be explained by the presence of a  $6\beta$ -OH group in **7** and **8**. A ROESY experiment led to the same conclusion with the observation of interactions between H-6 and CH<sub>3</sub>-28, and between H-4 and H-19 *endo*, thus supporting the configurations of C-4, C-5, and C-6. Consequently, the structures of **7** and **8** were respectively determined to be  $6\beta$ -hydroxyneoboutomellerone and  $6\beta$ -hydroxy-22-de-*O*-acetylneoboutomellerone. Along with compound **7**, another isomer (**9**) was isolated, in which either CH<sub>3</sub>-18 or CH<sub>3</sub>-30 was replaced by a hydroxymethylene function. No HMBC correlations were observed between this CH<sub>2</sub> and the backbone atoms, but the remaining methyl singlet displayed four long-range CH correlations, including one with CH<sub>2</sub>-15 (identified by scalar coupling with H-16). The OH was therefore placed at C-18, and this accounted for the chemical shift of the remaining CH<sub>3</sub> at  $\delta_{\text{H}}$  1.00 (CH<sub>3</sub>-18 at  $\delta_{\text{H}}$  >1.15, CH<sub>3</sub>-30 at  $\delta_{\text{H}}$  <1). Compound **9** was thus assigned the structure of 18-hydroxyneoboutomellerone.

Compound **10** appeared to belong to the same series as **1**, displaying NMR signals for two *O*-acetyl groups, the  $\gamma,\delta$ -cyclopropyl- $\alpha,\beta$ -unsaturated carbonyl, and the full and unmodified side chain. The HRMS indicated a C<sub>34</sub>H<sub>46</sub>O<sub>8</sub> composition corresponding to the formula of compound **7** plus one degree of unsaturation. Analysis of the <sup>1</sup>H NMR data was facilitated by the absence of signal overlap, with the sequence CH<sub>3</sub>-28, H-4, H-5, H-6, H-7, H-8 giving rise to well-separated signals, in which H-6 and H-7 appeared as a doublet

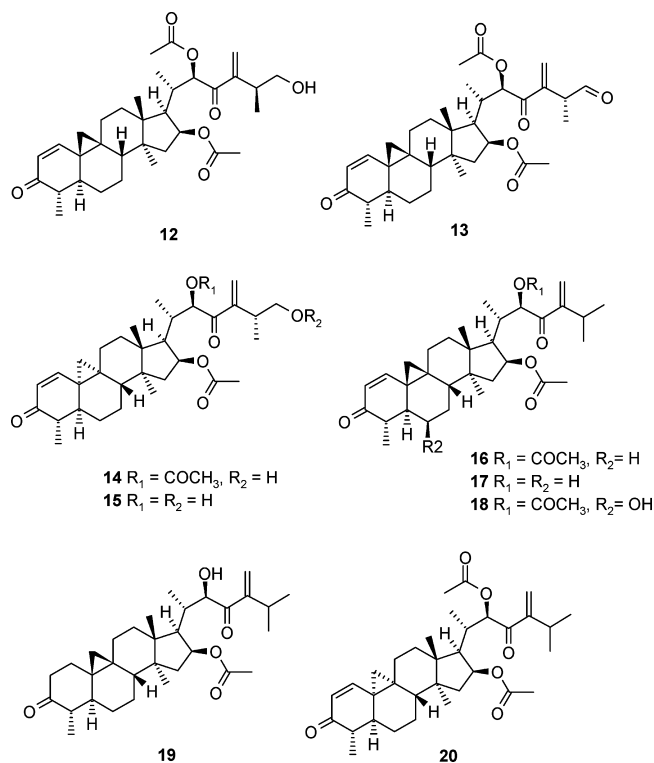
of doublets at  $\delta_{\text{H}}$  3.15 and 3.00. These two protons were attached to carbon atoms resonating at  $\delta_{\text{C}}$  52.8 and 55.0, respectively, suggesting that they were incorporated in an epoxide ring. In this particular compound, H-5 was distinguished as an isolated broad doublet with  $J = 11.3$  Hz ( $\delta_{\text{H}}$  2.45), thus proving its axial position and the *trans*-fusion of the A/B rings. The cyclopropane *endo* proton showed an Overhauser interaction with H-8 but not with H-6/7, allowing assignment of a  $\beta$ -configuration for the epoxide moiety. Shielding of the cyclopropane *exo*-proton, possibly as a result of interaction with the epoxide, is noteworthy. Compound **10** is thus  $6\beta,7\beta$ -oxidoneoboutomellerone.

The last compound in the “regular” series, **11**, had a  $\text{C}_{32}\text{H}_{48}\text{O}_7$  molecular formula, as established by HR-MS, indicative of a hydroxylated analogue of **2**. Absence of the AB system for H-1 and H-2 in the enone, as well as significant differences in the UV spectrum, suggested a 1,4-Michael addition of water to the enone functionality. An HMBC correlation between the *exo*-cyclopropane H-19 and the hydroxymethine allowed the hydroxy group to be placed at C-1. H-1 appeared as a broad triplet with  $J = 3$  Hz ( $\delta_{\text{H}}$  3.80), characteristic of an equatorial proton, and, consequently, the hydroxy group was deduced to be axial. This was confirmed by the observation of an NOE interaction between H-1 and the *exo*-cyclopropane proton. Compound **11** was thus identified as 1,2-dihydro-1 $\alpha$ -hydroxy-22-de-*O*-acetylneoboutomellerone.



At this point, all isolated compounds could be considered as being derived from **1** and **2** and thus were assigned the same configuration as evidenced by the series of related  $^{13}\text{C}$  NMR chemical shifts presented in Tables 1 and 2. Among the minor compounds, several isomers were isolated, including **12**, in which the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were almost superimposable with the corresponding spectra of **1**. The compounds were, however, quite different, with **12** being slightly less polar on reversed-phase HPLC. Since minor differences were observed for the signals of CH-25 and CH<sub>2</sub>-26, **12** was proposed to be the C-25 epimer of **1**, i.e., 25-*epi*-neoboutomellerone. This hypothesis was supported by the Dess-Martin oxidation of **1** into aldehyde **13**, which was found to be unstable, rapidly converting into isomers at C-25. Compound **14**, another isomer of **1**, gave a  $^{13}\text{C}$  NMR spectrum remarkably different from that of **1**, despite the presence of all the functionalities, i.e., the two enones, the cyclopropane, and the esters (see Table 2). The most significant chemical shift differences were found for the signals of ring B,

with C-7 being almost 7 ppm downfield (Table 2). In acetonitrile-*d*<sub>3</sub>, the  $^1\text{H}$  NMR spectrum displayed several well-separated signals, including H-17, H<sub>2</sub>-12, H-15, and *endo* H-19, while H-5 was masked by the broad acetonitrile residual signal, and *exo* H-19 partially overlapped with H<sub>3</sub>-30. A ROESY experiment showed a correlation between H-5 and the *endo* H-19, suggesting that these atoms were cofacial, thus offering two possibilities: H-5 $\beta$  in a “normal” series or H-5 $\alpha$  with H<sub>2</sub>-19  $\alpha$ -oriented. The presence of a *cis*-fused decalin moiety was deduced by the observation of an NOE correlation between H-1 and *exo* H-19, a feature not observed in any product in the *trans* series. The decision between the two hypotheses came in a serendipitous manner with the observation of a slow conversion of **1** into **14** upon storage in acetonitrile or by irradiation under a UV lamp, which could only be explained by opening of the cyclopropane (cleavage of the C-9/C-10 bond activated by the enone system) and ring closure to yield the thermodynamically more favorable *cis* isomer.<sup>13</sup> The configuration of all stereogenic centers was assumed to be as in compound **1**, and the proposed structure for **14** was 9,10-di-*epi*-25 $\xi$ -neoboutomellerone. Compound **15** is isomeric to **2** and was assigned the structure 9,10-di-*epi*-25 $\xi$ -22-de-*O*-acetylneoboutomellerone owing to the strong similarity between the  $^{13}\text{C}$  NMR spectra of **15** and **2**.



The next set of new compounds (**16**–**20**) shared the same side chain as before but without oxidation at C-26. As for **1** and **2**, the difference between **16** and **17** was an acetyl group at C-22. The absence of a deshielded signal for H-26 and the presence of two diastereotopic methyl doublets for H<sub>3</sub>-26 and H<sub>3</sub>-27 suggested that these compounds were 26-deoxyneoboutomellerone (**16**) and 22-de-*O*-acetyl-26-deoxyneoboutomellerone (**17**), respectively. Compound **18** belonged to the 22-*O*Ac series, but its molecular weight was 16 amu higher than observed for **16** ( $m/z$  591.3287 for  $[\text{M} + \text{Na}]^+$ ). The  $^1\text{H}$  NMR spectrum of **18** showed similarity to that of **7**, including a broad deshielded singlet for H-6 and characteristic deshielding for *endo* H-19



Table 2.  $^{13}\text{C}$  NMR Data of Compounds 9–16

carbon	9 <sup>a</sup>	10 <sup>b</sup>	11 <sup>b</sup>	12 <sup>a</sup>	13 <sup>b</sup>	14 <sup>a</sup>	15 <sup>a</sup>	16 <sup>b</sup>
1	153.5, CH	154.2, CH	74.0, CH	153.7, CH	155.5, CH	155.4, CH	155.7, CH	155.6, CH
2	128.3, CH	128.4, CH	49.3, CH <sub>2</sub>	128.3, CH	128.4, CH	126.5, CH	126.3, CH	128.4, CH
3	202.0, C	202.1, C	212.2, C	202.0, C	202.4, C	200.6, C	200.7, C	202.5, C
4	47.0, CH	45.6, CH	50.5, CH	46.9, CH	47.6, CH	47.7, CH	47.8, CH	47.6, CH
5	43.0, CH	41.5, CH	39.5, CH	42.5, CH	43.6, CH	39.4, CH	39.4, CH	43.6, CH
6	23.5, CH <sub>2</sub>	52.8, CH	26.1, CH <sub>2</sub>	23.4, CH <sub>2</sub>	24.3, CH <sub>2</sub>	20.6, CH <sub>2</sub>	20.6, CH <sub>2</sub>	24.3, CH <sub>2</sub>
7	24.0, CH <sub>2</sub>	55.0, CH	26.2, CH <sub>2</sub>	23.5, CH <sub>2</sub>	24.3, CH <sub>2</sub>	31.2, CH <sub>2</sub>	31.2, CH <sub>2</sub>	24.3, CH <sub>2</sub>
8	45.0, CH	38.9, CH	48.7, CH	44.3, CH	45.2, CH	40.7, CH	40.7, CH	45.2, CH
9	25.8, C	27.2, C	26.0, C	27.2, C	27.2, C	33.7, C	33.9, C	27.7, C
10	32.0, C	31.8, C	34.0, C	31.9, C	33.4, C	26.6, C	26.6, C	32.9, C
11	27.5, CH <sub>2</sub>	27.4, CH <sub>2</sub>	26.5, CH <sub>2</sub>	27.4, CH <sub>2</sub>	28.1, CH <sub>2</sub>	29.8, CH <sub>2</sub>	29.9, CH <sub>2</sub>	28.1, CH <sub>2</sub>
12	27.7, CH <sub>2</sub>	32.1, CH <sub>2</sub>	33.3, CH <sub>2</sub>	32.1, CH <sub>2</sub>	33.0, CH <sub>2</sub>	32.1, CH <sub>2</sub>	32.1, CH <sub>2</sub>	32.9, CH <sub>2</sub>
13	47.5, C	47.1, C	46.6, C	47.4, C	46.9, C	47.8, C	47.8, C	46.8, C
14	51.0, C	47.3, C	47.9, C	46.0, C	48.3, C	46.1, C	46.0, C	48.4, C
15	46.2, CH <sub>2</sub>	44.7, CH <sub>2</sub>	47.9, CH <sub>2</sub>	46.0, CH <sub>2</sub>	46.7, CH <sub>2</sub>	43.8, CH <sub>2</sub>	44.0, CH <sub>2</sub>	46.7, CH <sub>2</sub>
16	76.6, CH	76.3, CH	77.5, CH	75.9, CH	76.8, CH	76.0, CH	76.9, CH	76.6, CH
16a	169.4, C	171.3, C	171.3, C	170.1, C	171.2, C	170.1, C	170.1, C	171.3, C
16b	21.6, CH <sub>3</sub>	22.1, CH <sub>3</sub>	22.1, CH <sub>3</sub>	21.7, CH <sub>3</sub>	22.1, CH <sub>3</sub>	21.7, CH <sub>3</sub>	21.8, CH <sub>3</sub>	22.1, CH <sub>3</sub>
17	49.6, CH	49.7, CH	51.5, CH	50.3, CH	51.2, CH	49.5, CH	49.3, CH	51.3, CH
18	64.9, CH <sub>2</sub>	15.2, CH <sub>3</sub>	19.6, CH <sub>3</sub>	17.8, CH <sub>3</sub>	18.3, CH <sub>3</sub>	15.1, CH <sub>3</sub>	15.3, CH <sub>3</sub>	18.2, CH <sub>3</sub>
19	28.5, CH <sub>2</sub>	22.1, CH <sub>2</sub>	28.1, CH <sub>2</sub>	27.0, CH <sub>2</sub>	27.6, CH <sub>2</sub>	31.3, CH <sub>2</sub>	31.4, CH <sub>2</sub>	27.7, CH <sub>2</sub>
20	32.8, CH	33.2, CH	36.3, CH	32.2, CH	33.8, CH	32.5, CH	35.9, CH	33.0, CH
21	13.4, CH <sub>3</sub>	13.7, CH <sub>3</sub>	12.2, CH <sub>3</sub>	12.7, CH <sub>3</sub>	13.4, CH <sub>3</sub>	13.2, CH <sub>3</sub>	12.1, CH <sub>3</sub>	13.2, CH <sub>3</sub>
22	77.4, CH	78.6, CH	75.8, CH	77.8, CH	78.2, CH	77.7, CH	75.0, CH	78.5, CH
22a	170.5, C	171.7, C		170.7, C	171.6, C	170.6, C		171.7, C
22b	20.7, CH <sub>3</sub>	20.9, CH <sub>3</sub>		20.7, CH <sub>3</sub>	20.9, CH <sub>3</sub>	20.6, CH <sub>3</sub>		20.9, CH <sub>3</sub>
23	198.5, C	199.6, C	205.6, C	198.6, C	198.3, C	198.8, C	204.6, C	199.5, C
24	149.3, C	150.3, C	149.1, C	149.2, C	145.8, C	149.3, C	148.0, C	154.3, C
24a	123.6, CH <sub>2</sub>	124.7, CH <sub>2</sub>	126.5, CH <sub>2</sub>	124.3, CH <sub>2</sub>	128.6, CH <sub>2</sub>	123.7, CH <sub>2</sub>	125.6, CH <sub>2</sub>	122.3, CH <sub>2</sub>
25	37.4, CH	38.0, CH	37.6, CH	37.9, CH	48.9, CH	37.2, CH	36.7, CH	29.7, CH
26	66.8, CH <sub>2</sub>	66.4, CH <sub>2</sub>	66.7, CH <sub>2</sub>	66.8, CH <sub>2</sub>	201.7, CH	66.9, CH <sub>2</sub>	67.0, CH <sub>2</sub>	21.6, CH <sub>3</sub>
27	16.4, CH <sub>3</sub>	17.2, CH <sub>3</sub>	17.2, CH <sub>3</sub>	15.5, CH <sub>3</sub>	13.8, CH <sub>3</sub>	16.3, CH <sub>3</sub>	16.4, CH <sub>3</sub>	22.4, CH <sub>3</sub>
28	10.8, CH <sub>3</sub>	11.3, CH <sub>3</sub>	11.0, CH <sub>3</sub>	10.8, CH <sub>3</sub>	11.3, CH <sub>3</sub>	12.2, CH <sub>3</sub>	12.2, CH <sub>3</sub>	11.3, CH <sub>3</sub>
30	21.7, CH <sub>3</sub>	19.6, CH <sub>3</sub>	20.7, CH <sub>3</sub>	19.6, CH <sub>3</sub>	20.0, CH <sub>3</sub>	18.6, CH <sub>3</sub>	18.6, CH <sub>3</sub>	20.0, CH <sub>3</sub>

<sup>a</sup>In CDCl<sub>3</sub>. <sup>b</sup>In CD<sub>3</sub>CN.

( $\delta_{\text{H}}$  1.81). This feature, as well as the overall concordance between the spectra of **7** and **18**, indicated that **18** was 6 $\beta$ -hydroxy-26-deoxyneoboutomellerone. Compound **19**, like the preceding compounds, was characterized by four methyl doublets and therefore lacked oxidation at the terminus of the side chain. This compound had a molecular weight of 512, corresponding to a C<sub>32</sub>H<sub>48</sub>O<sub>5</sub> composition. This confirmed the absence of an oxygen atom and suggested that **19** was a dihydro analogue of a derivative of **2**. Since the characteristic signals for the ring A double bond were missing, it was deduced that **19** was 1,2-dihydro-22-de-O-acetyl-26-deoxyneoboutomellerone. Compound **20** was an isomer of **16** (C<sub>34</sub>H<sub>48</sub>O<sub>6</sub>), and, given the previous analyses, it was readily identified as the cyclopropyl isomer of **16**, i.e., 9,10-di-epi-26-deoxyneoboutomellerone.

Compounds **21**–**23** differed from the previous compounds by the absence of the C-24 *exo*-methylene group. Examination of the NMR spectra showed that C-24a was replaced by a proton ( $\delta_{\text{H}}$  6.15  $\pm$  0.2) and that the chain was terminated by a 3-methyl-2-butenoyl moiety. In these three compounds, the tetracyclic cycloartane core was intact, and, as for compounds **1** and **2**, **21** and **22** differed only by the presence of a C-22-*O*-acetyl moiety. Compound **21** was thus 24a-nor-24,25-didehydro-26-deoxyneoboutomellerone, and **22** was 22-de-*O*-acetyl-24a-nor-24,25-didehydro-26-deoxyneoboutomellerone. While **21** and

**22** were shown by  $^{13}\text{C}$  NMR to belong to the normal A/B *trans* series, **23** displayed a markedly different spectrum, characteristic of the *cis*  $\alpha$ -series. Consequently, its structure was deduced to be 9,10-di-epi-24a-nor-24,25-didehydro-26-deoxyneoboutomellerone (see Table 3).

Compounds **24** and **25** had molecular weights of 412 and 428, respectively, corresponding to molecular formulas C<sub>26</sub>H<sub>36</sub>O<sub>4</sub> and C<sub>26</sub>H<sub>36</sub>O<sub>5</sub>. Although six carbon atoms were missing, the  $^1\text{H}$  NMR spectra of both compounds showed strong resemblance with those of the neoboutomellerones and displayed signals for an acetate, an  $\alpha,\beta$ -unsaturated carbonyl, a cyclopropane, and the angular methyl groups. Initially, it was proposed that these compounds were lacking C-23 to C-27 of the “regular” series side chain. A formyl proton doublet at  $\delta_{\text{H}}$  9.58 ( $J = 1.8$  Hz) coupling with a doublet of doublets of quartets ( $\delta_{\text{H}}$  2.86) suggested that truncation had happened between C-22 and C-23. Since the  $^{13}\text{C}$  NMR spectrum showed that the tetracyclic core was intact, **24** was proposed to be 23,24,24a,25,26,27-hexa-nor-neoboutomelleron-22-al. This was confirmed by the high-yield oxidation of **2** into **24** by MnO<sub>2</sub>. It is worth noting that **1** was recovered unchanged under the same conditions. The  $^{13}\text{C}$  NMR spectra of **24** and **25** could barely be distinguished except for the formyl carbonyl carbon, which was replaced by a signal at  $\delta_{\text{C}}$  182.0. It was deduced that **25** was the corresponding acid

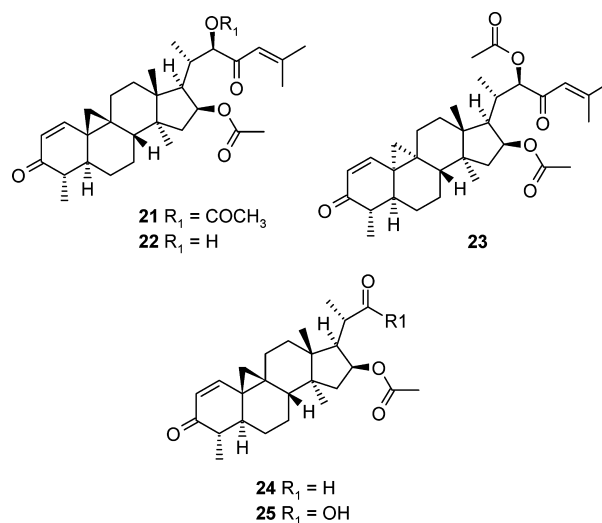
Table 3.  $^{13}\text{C}$  NMR Data of Compounds 17–24

carbon	17 <sup>a</sup>	18 <sup>a</sup>	19 <sup>a</sup>	20 <sup>a</sup>	21 <sup>a</sup>	22 <sup>a</sup>	23 <sup>a</sup>	24 <sup>a</sup>
1	155.7, CH	156.2, CH	33.5, CH <sub>2</sub>	157.3, CH	155.7, CH	155.7, CH	157.3, CH	155.4, CH
2	128.3, CH	127.5, CH	41.5, CH <sub>2</sub>	126.5, CH	128.4, CH	128.4, CH	126.5, CH	128.5, CH
3	202.5, C	203.4, C	213.3, C	201.0, C	202.6, C	202.5, C	201.0, C	202.3, C
4	47.6, CH	44.9, CH	50.5, CH	48.5, CH	47.6, CH	47.6, CH	48.5, CH	47.6, CH
5	43.6, CH	46.6, CH	47.0, CH	40.4, CH	43.5, CH	43.5, CH	40.4, CH	43.3, CH
6	24.3, CH <sub>2</sub>	65.6, CH	26.5, CH <sub>2</sub>	21.3, CH <sub>2</sub>	24.2, CH <sub>2</sub>	24.3, CH <sub>2</sub>	21.3, CH <sub>2</sub>	24.1, CH <sub>2</sub>
7	24.3, CH <sub>2</sub>	32.7, CH <sub>2</sub>	26.2, CH <sub>2</sub>	31.9, CH <sub>2</sub>	24.2, CH <sub>2</sub>	24.2, CH <sub>2</sub>	31.9, CH <sub>2</sub>	24.0, CH <sub>2</sub>
8	45.3, CH	41.1, CH	48.6, CH	41.5, CH	44.9, CH	45.0, CH	41.4, CH	44.3, CH
9	27.2, C	26.5, C	27.4, C	27.7, C	27.0, C	27.1, C	27.6, C	27.3, C
10	33.0, C	30.8, C	33.5, C	34.9, C	32.9, C	32.8, C	31.5, C	33.0, C
11	28.1, CH <sub>2</sub>	27.7, CH <sub>2</sub>	27.4, CH <sub>2</sub>	30.4, CH <sub>2</sub>	28.0, CH <sub>2</sub>	28.1, CH <sub>2</sub>	30.4, CH <sub>2</sub>	28.0, CH <sub>2</sub>
12	33.1, CH <sub>2</sub>	33.1, CH <sub>2</sub>	33.5, CH <sub>2</sub>	32.9, CH <sub>2</sub>	32.7, CH <sub>2</sub>	32.9, CH <sub>2</sub>	32.7	32.6, CH <sub>2</sub>
13	46.7, C	47.1, C	46.7, C	47.0, C	46.7, C	46.2, C	46.8, C	46.5, C
14	48.3, C	47.7, C	47.9, C	48.8, C	48.7, C	48.2, C	48.8, C	48.4, C
15	46.9, CH <sub>2</sub>	47.5, CH <sub>2</sub>	47.8, CH <sub>2</sub>	44.6, CH <sub>2</sub>	46.1, CH <sub>2</sub>	46.3, CH <sub>2</sub>	44.1, CH <sub>2</sub>	45.3, CH <sub>2</sub>
16	77.3, CH	76.8, CH	77.5, CH	76.5, CH	76.1, CH	76.5, CH	76.1, CH	75.1, CH
16a	171.3, C	171.3, C	171.3, C	171.3, C	171.3, C	171.3, C	171.3, C	170.9, C
16b	22.1, CH <sub>3</sub>	22.1, CH <sub>3</sub>	22.1, CH <sub>3</sub>	22.1, CH <sub>3</sub>	21.8, CH <sub>3</sub>	21.8, CH <sub>3</sub>	21.8, CH <sub>3</sub>	21.3, CH <sub>3</sub>
17	51.4, CH	51.5, CH	51.6, CH	50.6, CH	51.1, CH	51.2, CH	50.2, CH	50.7, CH
18	18.5, CH <sub>3</sub>	19.4, CH <sub>3</sub>	19.5, CH <sub>3</sub>	15.4, CH <sub>3</sub>	18.1, CH <sub>3</sub>	18.3, CH <sub>3</sub>	15.4, CH <sub>3</sub>	18.4, CH <sub>3</sub>
19	27.7, CH <sub>2</sub>	33.0, CH <sub>2</sub>	28.0, CH <sub>2</sub>	31.6, CH <sub>2</sub>	27.4, CH <sub>2</sub>	27.4, CH <sub>2</sub>	31.6, CH <sub>2</sub>	26.9, CH <sub>2</sub>
20	36.2, CH	33.0, CH	36.2, CH	33.0, CH	32.7, CH	34.4, CH	32.8, CH	45.1, CH
21	12.3, CH <sub>3</sub>	13.1, CH <sub>3</sub>	12.2, CH <sub>3</sub>	13.6, CH <sub>3</sub>	13.2, CH <sub>3</sub>	12.2, CH <sub>3</sub>	13.5, CH <sub>3</sub>	13.4, CH <sub>3</sub>
22	75.8, CH	78.5, CH	75.7, CH	78.6, CH	81.5, CH	79.0, CH	81.6, CH	205.2, CH
22a		171.7, C		171.7, C	171.7, C		171.6, C	
22b		20.9, CH <sub>3</sub>		20.9, CH <sub>3</sub>	21.2, CH <sub>3</sub>		21.2, CH <sub>3</sub>	
23	205.4, C	199.4, C	205.4, C	199.6, C	196.9, C	202.5, C	196.9, C	
24	153.1, C	154.3, C	153.1, C	154.3, C	120.5, CH	120.3, CH	120.5, CH	
24a	124.3, CH <sub>2</sub>	122.3, CH <sub>2</sub>	124.3, CH <sub>2</sub>	122.3, CH <sub>2</sub>				
25	29.5, CH	29.7, CH	29.5, CH	29.7, CH	159.8, C	159.9, C	159.7, C	
26	22.0, CH <sub>3</sub>	22.4, CH <sub>3</sub>	22.0, CH <sub>3</sub>	21.6, CH <sub>3</sub>	28.0, CH <sub>3</sub>	28.1, CH <sub>3</sub>	28.0, CH <sub>3</sub>	
27	22.6, CH <sub>3</sub>	21.7, CH <sub>3</sub>	22.6, CH <sub>3</sub>	22.4, CH <sub>3</sub>	21.0, CH <sub>3</sub>	21.3, CH <sub>3</sub>	21.0, CH <sub>3</sub>	
28	11.3, CH <sub>3</sub>	11.0, CH <sub>3</sub>	11.3, CH <sub>3</sub>	12.6, CH <sub>3</sub>	11.3, CH <sub>3</sub>	11.3, CH <sub>3</sub>	12.6, CH <sub>3</sub>	11.3, CH <sub>3</sub>
30	20.1, CH <sub>3</sub>	20.7, CH <sub>3</sub>	20.8, CH <sub>3</sub>	18.9, CH <sub>3</sub>	19.8, CH <sub>3</sub>	19.9, CH <sub>3</sub>	18.8, CH <sub>3</sub>	19.4, CH <sub>3</sub>

<sup>a</sup>In CD<sub>3</sub>CN.

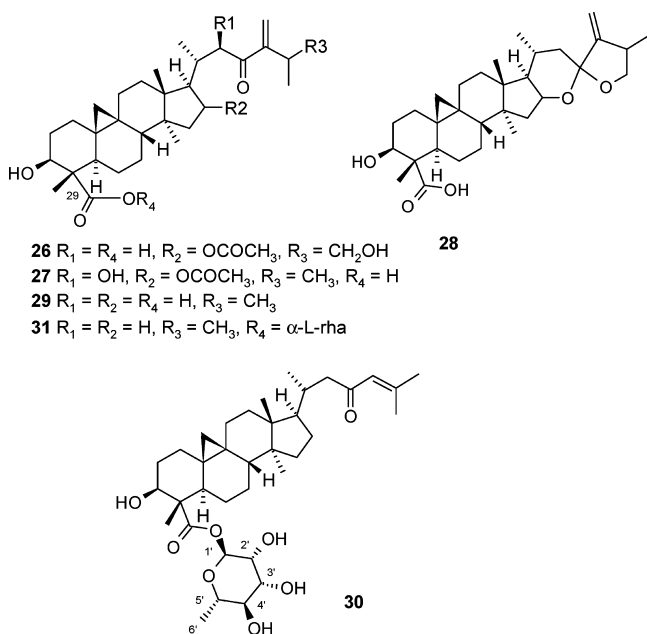
of **24**, i.e., 23,24,24a,25,26,27-hexa-nor-neoboutomelleron-22-oic acid.

Six more compounds (**26**–**31**) were isolated and, although devoid of any biological activity (*vide infra*), are interesting from a biosynthetic standpoint. They all shared common functionalities in rings A and B: a hydroxy group at C-3 and a quaternary C-4 bearing a hydroxycarbonyl or ester function. These compounds could be considered as the precursors for the aforementioned compounds, undergoing oxidation of a C-4 methyl group of a cycloartane, oxidation of C-3, followed by decarboxylation and introduction of the  $\Delta^{1,2}$  double bond, paralleling the well-known biosynthesis of the steroids. All six compounds had a 3*S* configuration (3 $\beta$ -OH) based on the large vicinal coupling between H-3 and H-2 $\alpha$  (ca. 11 Hz) and a 4*S* configuration to account for the strong shielding of the axial C-28 methyl group ( $\delta_{\text{C}}$  ca. 10). In the  $^1\text{H}$  NMR spectrum of **27**, H-5 was observed as a doublet of doublets with  $J = 12.2$  and 3.4 Hz and was therefore  $\alpha$ -axially oriented. Configurations of the other ring junctions, and of C-16, C-20, and C-22, were most likely the same as for the major compounds given the similarities in chemical shift values. Compounds **26** and **27** were isomers with a C<sub>33</sub>H<sub>50</sub>O<sub>7</sub> molecular formula, and comparison of their NMR spectra showed that they differed in the location of the side chain hydroxy group: C-22 for **27** and C-25 for **26**.



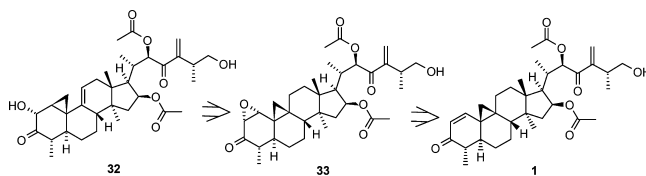
However, it was not possible to define the configuration of C-25 in compound **26** due to a paucity of material. Compound **29** was not oxidized at C-16 nor C-22, as shown by the presence of two methylene resonances at  $\delta_{\text{C}}$  29.3 and 46.5. The C-22 methylene group gave rise to two well-separated doublets of doublets, with 15.6 Hz geminal couplings and vicinal couplings

of 3.1 and 10.1 Hz, the smaller constant being close to that observed in all the HO-22 derivatives. The mass spectrum of compound **28** indicated a molecular formula of  $C_{31}H_{46}O_5$ , corresponding to the loss of HOAc with regard to **26** and **27**. Accordingly, no signal was observed for an acetate group in the  $^1H$  NMR spectrum. A noteworthy feature of the  $^{13}C$  NMR spectrum was the presence of a quaternary carbon at  $\delta_C$  108, which showed HMBC couplings with two *exo*-methylene protons,  $sp^2$  C-24a, and an oxymethylene corresponding to C-26. These characteristics were best accounted for by a ketal derived from a precursor possessing a C-23 carbonyl group and hydroxy groups at C-16 and C-26. No NOE correlations between protons of the spiroketal rings were evident, and we could not assign configurations to C-23 and C-25. Compounds **30** and **31** shared with **29** the absence of oxidation of C-16 and C-22, but analysis of the NMR spectra suggested that these highly polar compounds were glycosides. In both compounds, the sugar was tentatively identified as an  $\alpha$ -L-rhamnose. The difference between the two compounds was in the side chain, with **31** having the same side chain as **16** and **17**, and **30** having the truncated chain of **21** and **22**. Therefore, the respective structures of these compounds were 16-acetyl-3 $\beta$ ,26-dihydroxy-24-methyl-25 $\xi$ -cycloart-24(24a)-en-23-on-29-oic acid (**26**), 16-acetyl-3 $\beta$ ,22 $\beta$ -dihydroxy-24-methylcycloart-24(24a)-en-23-on-29-oic acid (**27**), 3 $\beta$ ,16 $\beta$ ,22 $\beta$ -trihydroxy-24-methyl-(16,23:23,26)-diepoxycycloart-24(24a)-en-29-oic acid (**28**), 3 $\beta$ -hydroxy-24-methylcycloart-24(24a)-en-23-on-29-oic acid (**29**), 3 $\beta$ -hydroxy-29-O-( $\alpha$ -L-rhamnopyranosyl)cycloart-24-en-23-on-29-oic acid (**30**), and 3 $\beta$ -hydroxy-29-O-( $\alpha$ -L-rhamnopyranosyl)-24-methylcycloart-24(24a)-en-23-on-29-oic acid (**31**).



Compound **32** was an isomer of **7**, with its  $^1H$  NMR spectrum displaying all the signals corresponding to rings C and D and of the side chain. However, the enoyl AB system in **7** was missing and the usual upfield doublet for H-19 was replaced by a doublet of doublets at  $\delta_H$  0.41 ( $J = 9.9, 4.7$  Hz) and 0.94 [broad triplet ( $J = 4.7$  Hz)], with both signals coupled to a broadened doublet of doublets at  $\delta_H$  1.88 ( $J = 9.9, 4.7$  Hz). The values of the vicinal coupling constants in the cyclopropane ring indicated that the most upfield proton was *endo*, since it is known that in such systems  $J_{trans} > J_{cis}$ .<sup>14</sup> A trisubstituted C-9/C-11 double

bond was evident, with H-11 being a broad doublet coupling with H<sub>2</sub>-12. This assignment was supported by an HMBC correlation to C-18. A broad hydroxymethine singlet [ $\delta_H$  4.16 ( $\delta_C$  73.2)] showed HMBC couplings with the methine, the methylene, and the quaternary carbon of the cyclopropane moiety. The COSY experiment showed a weak but definite coupling between this proton and the cyclopropane proton at  $\delta_H$  1.88. Consequently, the hydroxy group was placed at C-2, and the cyclopropane bridge between C-1 and C-10. A NOESY experiment showed a correlation between H-2 and the *exo*-cyclopropane proton, suggesting that this proton and the cyclopropane moiety were  $\beta$ -cofacially oriented, and therefore the 2-OH group must be  $\alpha$ -oriented. This molecule is another member of a rare family of triterpenes that are linked to the parent compounds through acidic rearrangements, for example, simplexol and *O*-methylcinicimol, arising from cimigenol.<sup>15</sup> In our case, the putative epoxide **33**, an oxidation product of **1**, should be the intermediate between **32** and the regular cycloartanes, and the rearrangement should be favored by an *anti* relationship between the cyclopropane and the oxirane, hence giving further support to the 2 $\alpha$ -OH configuration. On the basis of the above evidence, the structure of **32** was proposed to be 16,22-diacetyl-2,26-dihydroxy-29-nor-24-methyl-19(9 $\rightarrow$ 1)-*abeo*-cycloart-9(11),24(24a)-diene-3,23-diene.



**Structure–Activity Relationship Analysis.** Twenty-four of the isolated compounds the two non-natural derivatives, **4** and **13**, were evaluated in the cellular proteasome assay (Table 5). Cimiracemosides F and G,<sup>16</sup> cycloeucaleone,<sup>17</sup> 28-nor-cycloeucaleone, mangiferonic, and mangiferolic acids,<sup>18</sup> isolated from *Cimicifuga simplex*, *Musa sapientum*, and *Mangifera indica*, were also tested, but none were found to be active. Table 5 lists the induction factors (IFs) observed for the cycloartanes. The IF is defined as the increase in luciferase signal measured in the 4UB-Luc-DLD-1 cells, after 7 h exposure to the test compound, as compared to untreated cells.<sup>3b</sup> Epoxomicin was used as reference with an IF of 100 at 0.5  $\mu$ M. Due to the cytotoxicity of the compounds and/or the accumulation of proteasome substrates (known to produce cytotoxic effects), it was not possible to observe luminescence at the highest concentrations. It also explains why a maximum on the induction factor (IF) versus concentration plots is observed (bell-shaped curve). The two major compounds (**1**, **2**) from the plant were also the most active, with IF values of 48 and 45 at 1  $\mu$ M, respectively, and the activity decreased with the acylation level at the C-26 hydroxy group, with **1**, **2** > **3**. The integrity of the side chain seemed to be another requirement since compounds such as **4** or **24** showed no activity. Simple modifications, such as the removal of the *exo*-methylene in this side chain (**21** and **22**) or the alteration of CH<sub>3</sub>-18 into a hydroxymethylene (**9**), also reduced the biological activity. However, a C-6 hydroxy group (**7**, **8**), an epoxy moiety on C-6/C-7 (**10**), a configurational change at C-25 (**12**), a cyclopropyl isomerism (**14**, **15**), or substitutions at C-26 (**13**, **16**, **17**) represent structural modifications that do not significantly affect biological activity.

Table 4. <sup>13</sup>C NMR Data of Compounds 25–32

carbon	25 <sup>a</sup>	26 <sup>a</sup>	27 <sup>c</sup>	28 <sup>c</sup>	29 <sup>c</sup>	30 <sup>c</sup>	31 <sup>c</sup>	32 <sup>a</sup>
1	153.7, CH	31.4, CH <sub>2</sub>	32.8, CH <sub>2</sub>	32.8, CH <sub>2</sub>	32.8, CH <sub>2</sub>	32.7, CH <sub>2</sub>	32.7, CH <sub>2</sub>	23.6, CH
2	128.3, CH	29.4, CH <sub>2</sub>	30.5, CH <sub>2</sub>	30.5, CH <sub>3</sub>	30.5, CH <sub>2</sub>	30.4, CH <sub>2</sub>	30.4, CH <sub>2</sub>	73.2, CH
3	202.0, C	75.4, CH	76.3, CH	76.3, CH	76.3, CH	75.9, CH	75.9, CH	213.2, C
4	46.8, CH	54.5, C	56.0, C	55.9, C	55.8, C	56.5, C	56.5, C	40.4, CH
5	42.3, CH	44.0, CH	45.7, CH	45.5, CH	45.6, CH	46.0, CH	46.0, CH	47.6, CH
6	23.4, CH <sub>2</sub>	22.8, CH <sub>2</sub>	24.1, CH <sub>2</sub>	23.9, CH <sub>2</sub>	24.1, CH <sub>2</sub>	23.9, CH <sub>2</sub>	23.9, CH <sub>2</sub>	26.6, CH <sub>2</sub>
7	23.3, CH <sub>2</sub>	25.6, CH <sub>2</sub>	26.9, CH <sub>2</sub>	27.1, CH <sub>2</sub>	26.7, CH <sub>2</sub>	26.5, CH <sub>2</sub>	26.5, CH <sub>2</sub>	29.5, CH <sub>2</sub>
8	43.4, CH	47.7, CH	49.7, CH	49.1, CH	49.3, CH	49.3, CH	49.3, CH	44.2, CH
9	n.d. <sup>c</sup>	19.7, C	20.8, C	20.8, C	21.1, C	21.2, C	21.2, C	140.4, C
10	32.0, C	25.1, C	26.6, C	26.8, C	26.4, C	26.3, C	26.3, C	30.0, C
11	27.4, CH <sub>2</sub>	26.0, CH <sub>2</sub>	27.1, CH <sub>2</sub>	27.0, CH <sub>2</sub>	27.4, CH <sub>2</sub>	27.4, CH <sub>2</sub>	27.4, CH <sub>2</sub>	116.1, CH
12	32.0, CH <sub>2</sub>	32.3, CH <sub>2</sub>	33.8, CH <sub>2</sub>	34.1, CH <sub>2</sub>	34.0, CH <sub>2</sub>	33.9, CH <sub>2</sub>	33.9, CH <sub>2</sub>	36.2, CH <sub>2</sub>
13	45.2, C	45.9, C	46.9, C	45.7, C	46.6, C	46.6, C	46.6, C	45.1, C
14	47.5, C	47.1, C	48.3, C	47.4, C	50.1, C	50.1, C	50.1, C	45.2, C
15	44.0, CH <sub>2</sub>	45.7, CH <sub>2</sub>	48.1, CH <sub>2</sub>	44.7, CH <sub>2</sub>	36.5, CH <sub>2</sub>	36.5, CH <sub>2</sub>	36.5, CH <sub>2</sub>	45.0, CH <sub>2</sub>
16	75.0, CH	75.1, CH	78.1, CH	74.0, CH	29.3, CH <sub>2</sub>	29.3, CH <sub>2</sub>	29.3, CH <sub>2</sub>	76.2, CH
16a	171.8, C	171.0, C	172.3, C					170.1, C
16b	21.1, CH <sub>3</sub>	21.2, CH <sub>3</sub>	21.9, CH <sub>3</sub>					21.7, CH <sub>3</sub>
17	52.0, CH	54.7, CH	51.8, CH	57.7, CH	53.8, CH	53.8, CH	53.8, CH	49.1, CH
18	18.0, CH <sub>3</sub>	18.5, CH <sub>3</sub>	19.5, CH <sub>3</sub>	20.9, CH <sub>3</sub>	18.5, CH <sub>3</sub>	18.6, CH <sub>3</sub>	18.6, CH <sub>3</sub>	15.1, CH <sub>3</sub>
19	26.5, CH <sub>2</sub>	30.2, CH <sub>2</sub>	31.3, CH <sub>2</sub>	31.3, CH <sub>2</sub>	30.7, CH <sub>2</sub>	30.8, CH <sub>2</sub>	30.8, CH <sub>2</sub>	15.8, CH <sub>2</sub>
20	38.7, CH	27.3, CH	36.2, CH	26.8, CH	35.2, CH	34.9, CH	35.1, CH	32.4, CH
21	17.5, CH <sub>3</sub>	19.2, CH <sub>3</sub>	12.1, CH <sub>3</sub>	21.0, CH <sub>3</sub>	19.8, CH <sub>3</sub>	19.7, CH <sub>3</sub>	19.7, CH <sub>3</sub>	12.8, CH <sub>3</sub>
22	182.0, C	44.8, CH <sub>2</sub>	76.0, CH	43.8, CH <sub>2</sub>	46.5, CH <sub>2</sub>	52.7, CH <sub>2</sub>	46.4, CH <sub>2</sub>	77.6, CH
22a								170.6, C
22b								20.7, CH <sub>3</sub>
23		203.1, C	205.1, C	108.0, C	205.0, C	204.2, C	204.9, C	198.7, C
24		151.8, C	154.2, C	159.2, C	157.1, C	125.3, CH	157.1, C	149.3, C
24a		123.8, CH <sub>2</sub>	123.2, CH <sub>2</sub>	106.2, CH <sub>2</sub>	122.6, CH <sub>2</sub>		122.6, CH <sub>2</sub>	123.7, CH <sub>2</sub>
25		36.6, CH	29.9, CH	38.7, CH	29.1, CH	157.0, C	29.1, CH	37.2, CH
26		67.6, CH <sub>2</sub>	22.6, CH <sub>3</sub>	72.7, CH <sub>2</sub>	22.3, CH <sub>3</sub>	27.7, CH <sub>3</sub>	22.3, CH <sub>3</sub>	66.9, CH <sub>2</sub>
27		16.1, CH <sub>3</sub>	21.9, CH <sub>3</sub>	14.3, CH <sub>3</sub>	22.4, CH <sub>3</sub>	20.9, CH <sub>3</sub>	22.4, CH <sub>3</sub>	16.3, CH <sub>3</sub>
28	10.8, CH <sub>3</sub>	9.1, CH <sub>3</sub>	10.2, CH <sub>3</sub>	10.1, CH <sub>3</sub>	10.0, CH <sub>3</sub>	9.9, CH <sub>3</sub>	9.9, CH <sub>3</sub>	10.8, CH <sub>3</sub>
29		180.8, C	181.7, C	181.4, C	181.2, C	176.2, C	176.2, C	
30	19.0, CH <sub>3</sub>	20.0, CH <sub>3</sub>	20.7, CH <sub>3</sub>	20.0, CH <sub>3</sub>	19.7, CH <sub>3</sub>	19.7, CH <sub>3</sub>	19.8, CH <sub>3</sub>	19.4, CH <sub>3</sub>
1'						94.9, CH	94.9, CH	
2'						71.2, CH	71.2, CH	
3'						72.4, CH	72.4, CH	
4'						73.3, CH	73.3, CH	
5'						72.4, CH	72.4, CH	
6'						17.9, CH <sub>3</sub>	17.9, CH <sub>3</sub>	

<sup>a</sup>In CDCl<sub>3</sub>, <sup>c</sup>In methanol-*d*<sub>4</sub>. <sup>c</sup>n.d., not detected.

Moreover, minor changes in ring A were tolerated; the 1,2-dihydro derivatives (**5**, **6**) and 1-hydroxy derivative (**11**) showed some activity, possibly due to reoxidation *in vivo*. Compounds lacking the A-ring enone function (**26–28**, **30–32**) or the C-16-ester moiety (**4**, **28**) were also found to be weakly active. Acetylation of the C-22-hydroxy group results in minor decreases of activity among comparable structures (**1/2**, **5/6**, **7/8**, **16/17**, and **21/22**). Finally, enone functionalities associated with the side chain and in ring A appear to be crucial to maintain the biological activity, thus leaving the C-16-ester group and the C-22-hydroxy function as possible points of modification.

## CONCLUSION

From a phytochemical standpoint, it is not surprising to find cycloartanes as major secondary metabolites in a Euphorbiaceae

species such as *N. melleri*. cursory investigation of the terpenes from the twigs did not show the presence of cycloartanes but rather daphnanes, as observed in the only published investigation on similar material.<sup>6</sup> Despite the ubiquity of these triterpenes, the oxidation level of the molecules discussed here is most peculiar, with the rare combination of an  $\alpha,\beta$ -unsaturated carbonyl with a cyclopropane giving rise to photochemical reactivity. The two Michael acceptors in ring A and in the side chain add further biological properties to the neoboutomellerones. In fact, the two moieties may act as reversible or irreversible inhibitors across the ubiquitin–proteasome pathway. From a medicinal point of view, the novelty of the isolated compounds and their interesting pharmacological properties motivate the antitumor activity evaluation of the most abundant molecules **1** and **2**.<sup>19</sup>



Table 5. Induction Factors for Cycloartanes

compound	induction factor (4Ub Luc DLD-1 cell) at dose ( $\mu\text{M}$ )			
	10	5	1	0.5
1	4.4	37	48	2.1
2	2.9	28	45	1.4
3		48	12	1.1
4	2.6	1.0	0.9	
5	48	45	0.9	
6	77	10	1.2	
7		31	5.1	1.2
8		35	1.1	
9	1.1	12	1.2	
10	15	72	2.8	
11	74	11	1.6	1.3
12	60	71	1.5	1.0
13	31	70	1	
14	24	84	2	1.4
15		9.1	0.9	0.9
16	19	74	7.2	
17	20	65	2.4	
21	44	34	1.1	
22	53	5.2	1.1	
24	3.0	1.3	1.1	
26	2.8	1.3	1.0	
27	1.8	1.3	1.2	
28	1.3	1.3	1.2	
30	1.1	1.0	1.0	
31	1.1	1.0	1.1	
32	15	1.9	1.2	

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were determined on a Perkin-Elmer 341 automatic polarimeter. UV spectra were obtained in MeOH using a UV MC<sup>2</sup> Safas spectrophotometer. An FT-IR Bruker Tensor 27 spectrophotometer was used for scanning IR spectroscopy. The NMR spectra were recorded on a Bruker Avance II spectrometer equipped with a <sup>13</sup>C cryoprobe at 500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C; 2D experiments were performed using standard Bruker programs. The ESIMS and MS/MS were performed using a Bruker Esquire-LC ion trap mass spectrometer; the samples were introduced by infusion in a solution of MeOH. HR-ESIMS were obtained on a Bruker MicroTOF. TLC was carried out on precoated silica gel 60F<sub>254</sub> (Merck) with CH<sub>2</sub>Cl<sub>2</sub>–MeOH (97:3), and spots were visualized by heating after spraying with 3% H<sub>2</sub>SO<sub>4</sub> + 1% vanillin. CC was carried out on a prepacked Kieselgel cartridge (40–60  $\mu\text{m}$ ) with cyclohexane–EtOAc (50:50). Analytical HPLC was performed on a Merck-Hitachi apparatus equipped with an L-7200 automated sample injector, an L-7100 pump, a L-7450 diode array detector, a D-7000 interface, and Lachrom HSM or EZChrom software. A prepacked C<sub>18</sub> reversed-phase column (Lichrospher 100 RP-18, 4  $\times$  125 mm, 5  $\mu\text{m}$ ) was used for analytical HPLC with a binary gradient elution (solvent A: H<sub>2</sub>O; solvent B: MeCN) and a flow rate of 1 mL·min<sup>-1</sup>. Semipreparative HPLC was performed on an apparatus equipped with a VWR International LaPrep pump P110, a VWR LaPrep P314 Dual  $\lambda$  absorbance detector, and EZChrom software. A 50  $\times$  250 mm column (NW 50, Merck) with LiChroprep RP-18 (15–25  $\mu\text{m}$ ) and a prepacked C<sub>18</sub> reversed-phase column (Hibar-Lichrospher 100 RP-18, 25  $\times$  250 mm, 5  $\mu\text{m}$ ) were used for semipreparative HPLC with a binary gradient elution (solvent A: H<sub>2</sub>O; solvent B: MeCN) and a flow rate of 100 or 30 mL·min<sup>-1</sup>, and the chromatogram was monitored at 210 and 270 nm.

**Plant Material.** The original plant sample was collected by René Bellé (Pierre Fabre Research Institute) in Malantouen (Cameroon) on June 21, 1988, and an herbarium specimen was deposited under

reference RBL-273 in the Pierre Fabre Botanical Conservatory in Cambounet-sur-le-Sor (France). The plant was collected again by one of us (B.D.) at the same location and identified by comparison of the vouchers at the National Herbarium of the Museum National d'Histoire Naturelle in Paris. HPLC of the extracts showed similar profiles.

**Extraction and Isolation.** The dried leaves (1 kg) of *N. melleri* were powdered and extracted at room temperature with CH<sub>2</sub>Cl<sub>2</sub> (15 L) overnight. After filtration, the extract was concentrated under reduced pressure. The residue (45 g) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1 L) and stirred for 1 h with 100 g of activated vegetable charcoal. After filtration and concentration under reduced pressure, the extract (32 g) was partitioned between MeOH and cyclohexane. The MeOH fraction (15 g) was subjected to silica gel CC (600 g, 57  $\times$  420 mm) using cyclohexane–EtOAc (50:50) to give 50 fractions of 150 mL. All the fractions were analyzed by TLC on silica gel using the solvent mixture CH<sub>2</sub>Cl<sub>2</sub>–MeOH (97:3) and pooled according to TLC into four fractions (F1–F4). Fraction F1 (6.0 g) was purified by semipreparative HPLC RP-18 chromatography, eluting with a linear gradient (80 to 100% B), to give after repeated HPLC purification 17 (84 mg), 19 (53 mg), 23 (6 mg), 3 (5 mg), 22 (25 mg), 21 (18 mg), 16 (76 mg), 24 (7 mg), 20 (3 mg), and 18 (1 mg). Fractions F2 (5.5 g) and F3 (1.5 g) were purified by semipreparative RP-18 chromatography, eluting with a linear gradient (45–100% B). After repeated HPLC purification of F2, compounds 1 (2.5 g, 0.25%), 6 (180 mg, 0.018%), and 5 (107 mg, 0.0107%) were obtained. Fraction F3, after repeated HPLC purification, afforded the second most abundant compound, 2 (600 mg), 10 (11 mg), and 15 (63 mg). Fraction F4 (1.0 g) was purified by semipreparative RP-18 chromatography, to give after repeated HPLC purification with a linear gradient (90–100% B and 50–100% B) the most polar compounds, 26 (3 mg), 30 (5 mg), 27 (7 mg), 29 (23 mg), 28 (17 mg), 11 (12 mg), 25 (3 mg), 31 (2 mg), 7 (34 mg), 8 (18 mg), 14 (80 mg), 12 (5 mg), 32 (1 mg), and 9 (1 mg).

**Neoboutomellerone (1):** white fluffy solid,  $[\alpha]_{\text{D}}^{20}$  –55 (c 0.16, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 224 (3.77), 266 (3.83) nm; IR (film)  $\nu_{\text{max}}$  3430, 1735, 1665, 1602, 1230 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  6.85 (1H, d, J = 10.1 Hz, H-1), 6.13 (1H, s, H-24a), 6.02 (1H, d, J = 10.1 Hz, H-2), 5.89 (1H, br s, H-24a), 5.57 (1H, d, J = 2.1 Hz, H-22), 5.09 (1H, dt, J = 4.6, 7.6 Hz, H-16), 3.59 (2H, d, J = 5.8 Hz, 2 H-26), 2.88 (1H, sext, J = 6.5 Hz, H-25), 2.60 (1H, dq, J = 11.0, 7.0, 2.1 Hz, H-20), 2.31 (1H, dd, J = 14.0, 7.9 Hz, H-15a), 2.28 (1H, dd, J = 11.1, 7.5 Hz, H-17), 2.19 (1H, dq, J = 12.7, 7.9 Hz, H-4), 2.16 (3H, s, OAc-22), 2.08 (3H, s, OAc-16), 1.75–1.65 (3H, m, H-6, 2 H-12), 1.54 (1H, m, H-11), 1.47 (1H, m, H-7), 1.32 (1H, dd, J = 13.7, 4.6 Hz, H-15b), 1.20 (1H, d, J = 4.3 Hz, H-19a), 1.18 (3H, s, H-18), 1.12 (3H, d, J = 7.0 Hz, H-27), 1.10 (3H, d, J = 6.7 Hz, H-28), 0.95 (3H, s, H-30), 0.90 (3H, d, J = 6.7 Hz, H-21), 0.58 (1H, d, J = 4.3 Hz, H-19b); <sup>13</sup>C NMR, see Table 1; HR-ESIMS *m/z* 591.3297 (calcd for C<sub>34</sub>H<sub>48</sub>O<sub>7</sub>Na 591.3292).

**Preparation of Compound 1c.** To a stirred solution of 1 (53 mg, 0.093 mmol) in 1 mL of CH<sub>2</sub>Cl<sub>2</sub> were added DMAP (6 mg, 0.047 mmol, 0.5 equiv), phenyl isocyanate (11  $\mu\text{L}$ , 0.102 mmol, 1.1 equiv), and Et<sub>3</sub>N (11  $\mu\text{L}$ , 0.102 mmol, 1.1 equiv). The mixture was stirred at room temperature, and after 22 h, it was diluted with EtOAc and the organic solution was successively washed with 4% aqueous HCl, NaHCO<sub>3</sub>, and brine. The organic solution was dried over MgSO<sub>4</sub> and concentrated under vacuum. The residue was purified by chromatography on silica gel (elution: cyclohexane–EtOAc, 1:0 to 0:1) to give 1c as a white solid (30 mg, 47%): <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN)  $\delta$  7.68 (1H, br s, H-26b), 7.41 (2H, br d, J = 8.3 Hz, H-26d, H-26 h), 7.29 (2H, dd, J = 7.6, 8.3 Hz, H-26 g, H-26e), 7.03 (1H, tt, J = 7.6, 1.2 Hz, H-26f), 6.94 (1H, d, J = 10.1 Hz, H-1), 6.15 (1H, s, H-24a), 6.02 (1H, d, J = 0.6 Hz, H-24a), 5.90 (1H, d, J = 10.1 Hz, H-2), 5.54 (1H, d, J = 2.1 Hz, H-22), 5.09 (1H, dt, J = 4.4, 7.7 Hz, H-16), 4.13 (1H, dd, J = 10.7, 7.0 Hz, H-26a), 4.06 (1H, dd, J = 10.7, 6.4 Hz, H-26b), 3.07 (1H, sext, J = 7.0 Hz, H-25), 2.61 (1H, ddq, J = 2.3, 10.9, 7.0 Hz, H-20), 2.30 (1H, dd, J = 11.0, 7.3 Hz, H-17), 2.10 (3H, s, OAc-22), 2.03 (3H, s, OAc-16), 1.55 (1H, ddd, J = 6.6, 9.0, 14.8 Hz, H-11b), 1.37 (1H, dd, J = 13.9, 4.1 Hz, H-15b), 1.24 (1H, d, J = 4.6 Hz, H-19a), 1.18 (3H, s, H-18), 1.11 (3H, d, J = 7.0 Hz, H-27), 1.02

(3H, d,  $J = 6.7$  Hz, H-28), 0.95 (3H, s, H-30), 0.85 (3H, d,  $J = 7.0$  Hz, H-21), 0.57 (1H, d,  $J = 4.6$  Hz, H-19b);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{CN}$ )  $\delta$  202.4 (C-3), 198.8 (C-23), 171.8 (C-22a), 171.3 (C-16), 155.5 (C-1), 149.2 (C-24), 139.9 (C-26c), 129.9 (C-26e, 26 g), 128.4 (C-2), 125.7 (C-24a), 123.9 (C-26f), 119.5 (C-26d, 26 h), 78.5 (C-22), 76.7 (C-16), 68.5 (C-26), 51.3 (C-17), 48.3 (C-14), 47.6 (C-4), 46.8 (C-13), 46.7 (C-15), 45.2 (C-8), 43.6 (C-5), 35.0 (C-25), 33.4 (C-20), 32.9 (C-12), 28.1 (C-11), 27.6 (C-19), 27.2 (C-9), 24.3 (C-6, 7), 22.1 (C-16b), 21.0 (C-22b), 20.0 (C-30), 18.3 (C-18), 17.1 (C-27), 13.4 (C-21), 11.3 (C-28); ESIMS  $m/z$ : 688.24  $[\text{M} + \text{H}]^+$ .

**X-ray Crystallographic Analysis of Compound 1c.** Data were collected at low temperature (180 K) on a Bruker Kappa Apex II diffractometer using graphite-monochromated Mo  $K\alpha$  radiation ( $\lambda = 0.71073$  Å) and equipped with an Oxford Cryosystems Cryostream cooler device. The structure was solved by direct methods using SHELXS-86<sup>20</sup> and refined by means of least-squares procedures on  $F^2$  with the aid of the program SHELXL97<sup>20</sup> included in the software package WinGX version 1.63.<sup>21</sup> The atomic scattering factors were taken from International Tables for X-ray Crystallography.<sup>22</sup> All hydrogen atoms were placed geometrically and refined by using a riding model, except for the atoms H30A and H30B, which were located by Fourier differences. All non-hydrogen atoms were anisotropically refined, and in the last cycles of refinement, a weighting scheme was used, where weights were calculated from the following formula:  $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$  where  $P = (F_o^2 + 2F_c^2)/3$ . Drawing was performed with the program ORTEP32 with 30% probability displacement ellipsoids for non-hydrogen atoms.<sup>23</sup>

**Crystal Data for 1c:** orthorhombic,  $\text{C}_{41}\text{H}_{53}\text{NO}_8$ , space group  $P2_12_12_1$  with  $a = 11.5565(7)$  Å,  $b = 14.6892(10)$  Å,  $c = 22.0210(15)$  Å,  $V = 3738.2(4)$  Å<sup>3</sup>,  $Z = 4$ ,  $D_{\text{calc}} = 1.222$  g/cm<sup>3</sup>,  $m = 0.084$  mm<sup>-1</sup>,  $F(000) = 1480$ . Crystal size:  $0.35 \times 0.2 \times 0.08$  mm<sup>3</sup>. Independent reflections: 6975 with  $R_{\text{int}} = 0.0333$ . The final agreement factors are  $R1 = 0.0394$  and  $wR2 = 0.0972$  [ $I > 2\sigma(I)$ ]. Crystallographic data are deposited at the Cambridge Crystallographic Data center (deposition no. CCDC 826141).

**22-De-O-acetylneoboutomellerone (2):**  $[\alpha]_D^{20} -55$  (c 0.16,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 228 (3.71), 266 (3.86) nm; IR (film)  $\nu_{\text{max}}$  3453, 1725, 1661, 1601, 1245, 1043 cm<sup>-1</sup>;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.85 (1H, d,  $J = 10.1$  Hz, H-1), 6.18 (1H, s, H-24a), 6.02 (1H, s, H-24a), 5.99 (1H, d,  $J = 10.1$  Hz, H-2), 5.35 (1H, dt,  $J = 4.6, 7.5$  Hz, H-16), 4.74 (1H, dd,  $J = 5.5, 1.5$  Hz, H-22), 3.65 (2H, d,  $J = 6.1$  Hz, H-26), 3.52 (1H, d,  $J = 5.8$  Hz, OH-22), 2.96 (1H, sext,  $J = 6.7$  Hz, H-25), 2.52 (1H, dd,  $J = 11.0, 7.3$  Hz, H-17), 2.46 (1H, m, H-20), 2.35 (1H, dd,  $J = 14.0, 7.9$  Hz, H-15a), 2.19 (1H, dq,  $J = 12.5, 6.7$  Hz, H-4), 2.10 (3H, s, OAc-16), 2.05 (1H, m, H-11a), 1.99 (1H, dt,  $J = 5.2, 13.4$  Hz, H-5), 1.97 (1H, dd,  $J = 10.5, 6.9$  Hz, H-8a), 1.65 (1H, m, H-12a), 1.34 (1H, dd,  $J = 14.0, 4.4$  Hz, H-15b), 1.18 (1H, d,  $J = 4.5$  Hz, H-19a), 1.18 (3H, s, H-18), 1.14 (3H, d,  $J = 7.0$  Hz, H-27), 1.10 (3H, d,  $J = 7.0$  Hz, H-28), 0.99 (3H, s, H-30), 0.88 (1H, m, H-6b), 0.66 (3H, d,  $J = 7.0$  Hz, H-21), 0.57 (1H, d,  $J = 4.5$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 1; HR-ESIMS  $m/z$  549.3179 (calcd for  $\text{C}_{32}\text{H}_{46}\text{O}_6\text{Na}$  549.3187).

**Preparation of Mosher Ester Derivatives 1a, 1b, 2a, and 2b.** Compounds 1 and 2 (10 mg) in dry pyridine (2.0 mL) were transferred into an HPLC vial (2 mL) capped with a septum and were treated with 30  $\mu\text{L}$  (175  $\mu\text{mol}$ ) of (S)-(+)- $\alpha$ -methoxy- $\alpha$ -trifluoromethylphenylacetyl chloride (MTPA chloride). The mixtures were stirred at room temperature for 2 h. After concentration under reduced pressure, they were purified by semipreparative HPLC RP-18 chromatography, eluting with a linear gradient (55–100% B) to afford 1a and 2a. Derivatives 1b and 2b were prepared in a similar fashion by using (R)-(-)- $\alpha$ -methoxy- $\alpha$ -trifluoromethylphenylacetyl chloride.

**1-(R)-MTPA ester (1a):**  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  4.39 (1H, dd,  $J = 6.2, 10.9$  Hz, H-26a), 4.27 (1H, dd,  $J = 5.7, 10.9$  Hz, H-26b); ESIMS  $m/z$  807  $[\text{M} + \text{Na}]^+$ .

**1-(S)-MTPA ester (1b):**  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  4.34 (2H, AB part of an ABX system, H-26); ESIMS  $m/z$  807  $[\text{M} + \text{Na}]^+$ .

**2-(R)-MTPA ester (2a):**  $^1\text{H}$  NMR (methanol- $d_4$ , 500 MHz)  $\delta$  4.44 (1H, dd,  $J = 6.0, 10.9$  Hz, H-26a), 4.35 (1H, dd,  $J = 5.1, 10.9$  Hz, H-26b), 2.59 (1H, m, H-20); ESIMS  $m/z$  981  $[\text{M} + \text{Na}]^+$ .

**2-(S)-MTPA ester (2b):**  $^1\text{H}$  NMR (methanol- $d_4$ , 500 MHz)  $\delta$  4.40 (2H, m, H-26), 2.65 (1H, m, H-20); ESIMS  $m/z$  981  $[\text{M} + \text{Na}]^+$ .

**26-Acetylneoboutomellerone (3):** yellow gum;  $[\alpha]_D^{20} -61$  (c 0.05,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 222 (3.78), 266 (3.74) nm; IR (film)  $\nu_{\text{max}}$  3299, 2933, 2874, 1736, 1699, 1596, 1372, 1230, 1022 cm<sup>-1</sup>;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.94 (1H, d,  $J = 10.1$  Hz, H-1), 6.10 (1H, s, H-24a), 5.95 (1H, d,  $J = 0.6$  Hz, H-24a), 5.88 (1H, d,  $J = 10.1$  Hz, H-2), 5.52 (1H, d,  $J = 2.1$  Hz, H-22), 5.09 (1H, dt,  $J = 4.6, 7.6$  Hz, H-16), 4.04 (1H, dd,  $J = 7, 11$  Hz, H-26a), 3.99 (1H, dd,  $J = 6.5, 11$  Hz, H-26b), 3.00 (1H, sext,  $J = 6.9$  Hz, H-25), 2.57 (1H, ddq,  $J = 11.0, 2.1, 7.0$  Hz, H-20), 2.28 (1H, dd,  $J = 11.0, 7.6$  Hz, H-17), 2.08 (3H, s, OAc-22), 2.05 (3H, s, OAc-16), 1.96 (3H, s, OAc-26), 1.56 (1H, ddd,  $J = 6.3, 8.5, 15$  Hz, H-11b), 1.45 (1H, m, H-7a), 1.37 (1H, dd,  $J = 14.5, 4.5$  Hz, H-15b), 1.24 (1H, d,  $J = 4.6$  Hz, H-19a), 1.19 (1H, m, H-7b), 1.18 (3H, s, H-18), 1.07 (3H, d,  $J = 7.0$  Hz, H-27), 1.02 (3H, d,  $J = 6.7$  Hz, H-28), 0.95 (3H, s, H-30), 0.94 (1H, m, H-6b), 0.84 (3H, d,  $J = 7.0$  Hz, H-21), 0.57 (1H, d,  $J = 4.6$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 1; HR-ESIMS  $m/z$  633.3399 (calcd for  $\text{C}_{36}\text{H}_{50}\text{O}_8\text{Na}$  633.3398).

**Preparation of Compound 3.** To a stirred solution of compound 2 (50 mg, 0.088 mmol) in 4 mL of  $\text{CH}_2\text{Cl}_2$  at 0 °C were added pyridine (300  $\mu\text{L}$ , 3.7 mmol) and acetyl chloride (60  $\mu\text{L}$ , 0.88 mmol, 10 equiv). The mixture was stirred overnight, MeOH (0.5 mL) was added, and the mixture was concentrated under vacuum. The crude material was purified by chromatography on silica gel (elution: from cyclohexane–EtOAc, 7:3 to 6:4) to yield 3 (52 mg, 75%), identical by all means with the natural product.

**Preparation of 22,23,26-Trihydroxy-29-nor-24-methyl-16,22-epoxycycloarta-1,24(24a)-diene-3,23-dione (4).** To a stirred solution of compound 1 (20 mg, 0.038 mmol) in  $\text{CH}_3\text{CN}$  (300  $\mu\text{L}$ ) was added NaOH (570  $\mu\text{L}$ , 0.437 mmol,  $c = 1$  mol/L, 15 equiv). The mixture was stirred for 22 h at room temperature, then diluted with EtOAc, and filtered over Celite. After concentration under vacuum, the product was purified by chromatography on silica gel (elution: cyclohexane–EtOAc, 1:1) to obtain 4 as a translucent film (3 mg, 17%):  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.96 (1H, d,  $J = 10.1$  Hz, H-1), 5.91 (1H, d,  $J = 10.1$  Hz, H-2), 5.24 (1H, s, H-24a), 5.11 (1H, s, H-24a), 4.66 (1H, dt,  $J = 7.2, 8.5$  Hz, H-16), 3.97 (1H, d,  $J = 5.6$  Hz, H-23), 3.79 (1H, d,  $J = 5.6$  Hz, OH-23), 3.48 (1H, s, OH-22), 3.47 (1H, m, H-26a), 3.40 (1H, ddd,  $J = 9.6, 8.7, 4.6$  Hz, H-26b), 3.23 (1H, t,  $J = 4.7$  Hz, OH-26), 2.63 (1H, sext,  $J = 7.0$  Hz, H-25), 2.47 (1H, quint,  $J = 6.8$  Hz, H-20), 1.31 (1H, d,  $J = 4.6$  Hz, H-19a), 1.27 (1H, m, H-7b), 1.13 (3H, s, H-18), 1.03 (3H, d,  $J = 6.7$  Hz, H-28), 1.00 (3H, d,  $J = 7.0$  Hz, H-27), 0.95 (3H, d,  $J = 6.7$  Hz, H-21), 0.92 (3H, s, H-30), 0.50 (1H, d,  $J = 4.6$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 1; ESIMS  $m/z$  507.3  $[\text{M} + \text{Na}]^+$ , 991.6  $[2\text{M} + \text{Na}]^+$ .

**1,2-Dihydroxoneoboutomellerone (5):** yellow gum;  $[\alpha]_D^{20} +36$  (c 0.08,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 216 (3.78), 290 (2.63) nm; IR (film)  $\nu_{\text{max}}$  3513, 2965, 2871, 1734, 1705, 1452, 1376, 1231 cm<sup>-1</sup>;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.05 (1H, s, H-24a), 5.90 (1H, d,  $J = 0.9$  Hz, H-24a), 5.53 (1H, d,  $J = 2.1$  Hz, H-22), 5.08 (1H, dt,  $J = 4.3, 7.6$  Hz, H-16), 3.53 (1H, dt,  $J = 10.6, 5.8$  Hz, H-26a), 3.39 (1H, dt,  $J = 10.6, 6.1$  Hz, H-26b), 2.77 (1H, sext,  $J = 6.7$  Hz, H-25), 2.68 (1H, t,  $J = 5.6$  Hz, OH-26), 2.60 (1H, ddq,  $J = 10.7, 1.8, 6.7$  Hz, H-20), 2.43 (1H, td,  $J = 13.6, 6.4$  Hz, H-2a), 2.20 (1H, dd,  $J = 14.0, 7.9$  Hz, H-15a), 2.09 (3H, s, OAc-22), 2.11 (1H, m, H-11a), 2.03 (3H, s, OAc-16), 1.82 (1H, m, H-1a), 1.60–1.55 (2H, m, H-1b, H-5), 1.36 (1H, dd,  $J = 14.5, 4.1$  Hz, H-15b), 1.23 (3H, s, H-18), 1.10 (1H, m, H-7b), 1.03 (3H, d,  $J = 7.0$  Hz, H-27), 0.97 (3H, s, H-30), 0.91 (3H, d,  $J = 6.7$  Hz, H-28), 0.84 (3H, d,  $J = 7.0$  Hz, H-21), 0.75 (1H, dq,  $J = 2.4, 12.6$  Hz, H-6b), 0.65 (1H, br d,  $J = 4.0$  Hz, H-19a), 0.47 (1H, d,  $J = 4.0$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 1; HR-ESIMS  $m/z$  593.3451 (calcd for  $\text{C}_{34}\text{H}_{50}\text{O}_7\text{Na}$  593.3449).

**1,2-Dihydroxy-22-de-O-acetylneoboutomellerone (6):** yellow gum;  $[\alpha]_D^{20} +11$  (c 0.12,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 214 (3.74), 262 (3.23) nm; IR (film)  $\nu_{\text{max}}$  3435, 2932, 2873, 1731, 1705, 1700, 1452, 1376, 1237, 1022, 997 cm<sup>-1</sup>;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.12 (1H, s, H-24a), 5.99 (1H, d,  $J = 0.9$  Hz, H-24a), 5.20 (1H, dt,  $J = 4.4, 7.6$  Hz, H-16), 4.72 (1H, dd,  $J = 6.1, 2.1$  Hz, H-22), 3.56 (1H, dt,  $J = 10.5, 5.8$  Hz, H-26a), 3.54 (1H, d,  $J = 6.1$  Hz, OH-22), 3.41 (1H, dt,

$J = 10.5, 5.8$  Hz, H-26b), 2.83 (1H, sext,  $J = 6.7$  Hz, H-25), 2.67 (1H, t,  $J = 5.8$  Hz, OH-26), 2.07 (1H, m, H-11a), 2.03 (3H, s, OAc-16), 1.82 (1H, m, H-1a), 1.37 (1H, ddd,  $J = 14.2, 4.4, 0.9$  Hz, H-15b), 1.22 (3H, s, H-18), 1.12 (1H, dq,  $J = 2.7, 12.8$  Hz, H-7b), 1.05 (3H, d,  $J = 7.3$  Hz, H-27), 0.97 (3H, d,  $J = 0.6$  Hz, H-30), 0.91 (3H, d,  $J = 6.7$  Hz, H-28), 0.75 (1H, dq,  $J = 2.4, 12.5$  Hz, H-6b), 0.65 (1H, m, H-19a), 0.64 (3H, d,  $J = 6.4$  Hz, H-21), 0.47 (1H, d,  $J = 4.0$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 1; HR-ESIMS  $m/z$  551.3342 (calcd for  $\text{C}_{32}\text{H}_{48}\text{O}_6\text{Na}$  551.3343).

**6 $\beta$ -Hydroxyneoboutomellerone (7):** yellow gum;  $[\alpha]_{\text{D}}^{20} -43$  (c 0.13,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 212 (3.84), 220 (3.84), 266 (3.87) nm; IR (film)  $\nu_{\text{max}}$  3409, 2937, 2876, 1733, 1662, 1454, 1374, 1232, 1022  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.76 (1H, d,  $J = 10$  Hz, H-1), 6.14 (1H, s, H-24a), 5.96 (1H, d,  $J = 10$  Hz, H-2), 5.90 (1H, s, H-24a), 5.59 (1H, d,  $J = 2.1$  Hz, H-22), 5.10 (1H, dt,  $J = 4.4, 7.7$  Hz, H-16), 4.20 (1H, br s, H-6), 3.60 (2H, d,  $J = 5.8$  Hz, H-26), 2.89 (1H, sext,  $J = 6.4$  Hz, H-25), 2.35 (1H, dd,  $J = 14.0, 7.9$  Hz, H-15a), 2.20 (1H, m, H-11a), 2.16 (3H, s, OAc-22), 2.08 (3H, s, OAc-16), 1.99 (1H, m, H-5), 1.86 (1H, d,  $J = 3.7$  Hz, H-19a), 1.76 (2H, m, 2 H-12), 1.56 (1H, m, H-7a), 1.48 (1H, dt,  $J = 1.7, 13.3$  Hz, H-7b), 1.25 (3H, s, H-18), 1.24 (3H, d,  $J = 7.0$  Hz, H-28), 1.13 (3H, d,  $J = 7.0$  Hz, H-27), 1.0 (3H, s, H-30), 0.91 (3H, d,  $J = 6.7$  Hz, H-21), 0.72 (1H, d,  $J = 3.7$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 1; HR-ESIMS  $m/z$  607.3243 (calcd for  $\text{C}_{34}\text{H}_{48}\text{O}_8\text{Na}$  607.3241).

**6 $\beta$ -Hydroxy-22-de-O-acetylneoboutomellerone (8):** yellow gum;  $[\alpha]_{\text{D}}^{20} -8$  (c 0.14,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 210 (3.94), 266 (3.78) nm; IR (film)  $\nu_{\text{max}}$  3435, 2934, 2876, 1729, 1666, 1453, 1375, 1236, 1023  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.76 (1H, d,  $J = 10$  Hz, H-1), 6.18 (1H, s, H-24a), 6.03 (1H, s, H-24a), 5.94 (1H, d,  $J = 10$  Hz, H-2), 5.35 (1H, dt,  $J = 4.4, 7.1$  Hz, H-16), 4.74 (1H, s, H-22), 4.19 (1H, br s, H-6), 3.64 (2H, d,  $J = 6.1$  Hz, H-26), 3.59 (1H, d,  $J = 5.5$  Hz, OH-22), 2.96 (1H, sext,  $J = 6.4$  Hz, H-25), 2.58 (1H, m, H-4), 2.52 (1H, m, H-17), 2.45 (1H, m, H-20), 2.37 (1H, dd,  $J = 13.7, 7.9$  Hz, H-15a), 2.30 (1H, dd,  $J = 12.7, 4.4$  Hz, H-8), 2.22 (1H, m, H-11a), 2.09 (3H, s, OAc-16), 1.99 (1H, dd,  $J = 12.8, 2.4$  Hz, H-5), 1.84 (1H, d,  $J = 3.1$  Hz, H-19a), 1.75 (2H, m, 2 H-12), 1.58 (1H, dt,  $J = 4.4, 12.9$  Hz, H-7a), 1.48 (1H, t,  $J = 13.1$  Hz, H-7b), 1.40–1.20 (2H, m, H-15b, H-11b), 1.24 (3H, d,  $J = 7.3$  Hz, H-28), 1.23 (3H, br s, H-18), 1.14 (3H, d,  $J = 7.0$  Hz, H-27), 1.04 (3H, s, H-30), 0.70 (1H, d,  $J = 3.1$  Hz, H-19b), 0.67 (3H, d,  $J = 6.4$  Hz, H-21);  $^{13}\text{C}$  NMR, see Table 1; HR-ESIMS  $m/z$  565.3133 (calcd for  $\text{C}_{32}\text{H}_{46}\text{O}_7\text{Na}$  565.3136).

**18-Hydroxyneoboutomellerone (9):** yellow gum;  $[\alpha]_{\text{D}}^{20} -16$  (c 0.03,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 214 (3.14), 266 (2.96) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.80 (1H, d,  $J = 10$  Hz, H-1), 6.07 (1H, s, H-24a), 5.98 (1H, d,  $J = 10$  Hz, H-2), 5.86 (1H, br d, H-24), 5.57 (1H, d,  $J = 2.1$  Hz, H-22), 5.18 (1H, dt,  $J = 4.3, 7.6$  Hz, H-16), 4.06 (1H, m, H-18a), 3.94 (1H, d,  $J = 12.2$  Hz, H-18b), 3.58 (2H, m, 2 H-26), 2.86 (1H, sext,  $J = 7.0$  Hz, H-25), 2.72 (1H, m, H-20), 2.36 (1H, dd,  $J = 11.3, 7.6$  Hz, H-17), 2.28 (1H, dd,  $J = 14.3, 7.9$  Hz, H-15a), 2.19 (1H, m, H-4), 2.14 (3H, s, OAc-22), 2.09 (3H, s, OAc-16), 2.03 (2H, m, H-5, H-8), 1.85 (1H, m, H-12a), 1.70 (1H, m, H-6a), 1.63 (1H, m, H-12b), 1.60 (1H, m, H-15b), 1.35 (2H, m, H-7a, H-11b), 1.19 (1H, d,  $J = 4.3$  Hz, H-19a), 1.16 (1H, m, H-7b), 1.11 (3H, d,  $J = 7.3$  Hz, H-27), 1.09 (3H, d,  $J = 6.9$  Hz, H-28), 1.00 (3H, s, H-30), 0.93 (3H, d,  $J = 6.7$  Hz, H-21), 0.90 (1H, m, H-6b), 0.60 (1H, d,  $J = 4.3$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 2; HR-ESIMS  $m/z$  607.3216 (calcd for  $\text{C}_{34}\text{H}_{48}\text{O}_8\text{Na}$  607.3241).

**6 $\beta$ ,7 $\beta$ -Chloneoboutomellerone (10):** yellow gum;  $[\alpha]_{\text{D}}^{20} -86$  (c 0.16,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 224 (3.92), 262 (3.93) nm; IR (film)  $\nu_{\text{max}}$  3411, 2966, 2878, 1735, 1669, 1375, 1233, 1022  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.94 (1H, d,  $J = 10$  Hz, H-1), 6.06 (1H, s, H-24a), 5.91 (1H, d,  $J = 10.0$  Hz, H-2), 5.90 (1H, br s, H-24a), 5.54 (1H, d,  $J = 2.1$  Hz, H-22), 5.16 (1H, dt,  $J = 4.7, 7.7$  Hz, H-16), 3.53 (1H, dd,  $J = 10.4, 6.4$  Hz, H-26a), 3.39 (1H, dd,  $J = 10.4, 6.6$  Hz, H-26b), 3.15 (1H, dd,  $J = 4.3, 1.5$  Hz, H-6), 3.00 (1H, dd,  $J = 4.3, 1.8$  Hz, H-7), 2.83 (1H, br d,  $J = 1.2$  Hz, H-8), 2.77 (1H, sext,  $J = 6.7$  Hz, H-25), 2.70 (1H, br s, OH-26), 2.63 (1H, ddq,  $J = 10.8, 2.1, 7.0$  Hz, H-20), 2.55 (1H, dq,  $J = 12.5, 7.0$  Hz, H-4), 2.45 (1H, br d,  $J = 11.3$  Hz, H-5), 2.33 (1H, dd,  $J = 13.4, 7.9$  Hz, H-15a), 2.28 (1H, dd,  $J = 10.8, 7.8$  Hz, H-17), 2.08 (3H, s, OAc-22), 2.06 (3H, s, OAc-16), 2.04

(2H, m, H-11a, H-19a), 1.40 (1H, ddd,  $J = 15.9, 3.1, 1.6$  Hz, H-11b), 1.23 (3H, d,  $J = 7.0$  Hz, H-28), 1.20 (3H, s, H-18), 1.03 (3H, d,  $J = 7.0$  Hz, H-27), 0.94 (3H, s, H-30), 0.86 (3H, d,  $J = 7.0$  Hz, H-21), 0.03 (1H, d,  $J = 4.0$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 2; HR-ESIMS  $m/z$  605.3091 (calcd for  $\text{C}_{34}\text{H}_{46}\text{O}_8\text{Na}$  605.3085).

**1,2-Dihydro-1 $\alpha$ -hydroxy-22-de-O-acetylneoboutomellerone (11):** yellow gum;  $[\alpha]_{\text{D}}^{20} +30$  (c 0.12,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 216 (3.920) nm; IR (film)  $\nu_{\text{max}}$  3401, 2934, 2876, 1707, 1669, 1376, 1237, 1021  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.12 (1H, s, H-24a), 5.99 (1H, d,  $J = 0.9$  Hz, H-24a), 5.21 (1H, dt,  $J = 4.3, 7.5$  Hz, H-16), 4.72 (1H, dd,  $J = 5.3, 1.7$  Hz, H-22), 3.80 (1H, t,  $J = 3.1$  Hz, H-1), 3.55 (2H, dd,  $J = 10.9, 6.1$  Hz, H-26a + OH-22), 3.42 (1H, dd,  $J = 10.9, 7.7$  Hz, H-26b), 2.83 (1H, sext,  $J = 6.4$  Hz, H-25), 2.80 (1H, br s, OH-1), 2.69 (1H, br s, OH-26), 2.64 (1H, ddd,  $J = 14.0, 3.7, 0.5$  Hz, H-2a), 2.46 (1H, ddq,  $J = 11.0, 2.1, 6.7$  Hz, H-20), 2.42 (1H, dd,  $J = 11.0, 7.0$  Hz, H-17), 2.14 (1H, m, H-5), 2.03 (3H, s, OAc-16), 1.74 (1H, m, H-6a), 1.21 (3H, s, H-18), 1.05 (3H, d,  $J = 7.0$  Hz, H-27), 1.01 (3H, s, H-30), 0.92 (3H, d,  $J = 6.4$  Hz, H-28), 0.80 (1H, dq,  $J = 2.4, 12.6$  Hz, H-6b), 0.73 (1H, d,  $J = 4.3$  Hz, H-19a), 0.64 (3H, d,  $J = 6.6$  Hz, H-21), 0.48 (1H, d,  $J = 4.3$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 2; HR-ESIMS  $m/z$  567.3296 (calcd for  $\text{C}_{32}\text{H}_{48}\text{O}_7\text{Na}$  567.3292).

**25-Epi-neoboutomellerone (12):** yellow gum;  $[\alpha]_{\text{D}}^{20} +9$  (c 0.03,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 210 (3.54), 264 (3.54) nm; IR (film)  $\nu_{\text{max}}$  3386, 2927, 1732, 1667, 1570, 1375, 1233, 1022  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.82 (1H, d,  $J = 9.8$  Hz, H-1), 6.11 (1H, s, H-24a), 5.98 (1H, d,  $J = 9.8$  Hz, H-2), 5.83 (1H, d,  $J = 0.9$  Hz, H-24a), 5.52 (1H, d,  $J = 2.1$  Hz, H-22), 5.07 (1H, dt,  $J = 4.4, 7.7$  Hz, H-16), 3.56 (2H, t,  $J = 6.0$  Hz, 2 H-26), 2.96 (1H, sext,  $J = 6.7$  Hz, H-25), 2.59 (1H, m, H-20), 2.28 (1H, dd,  $J = 7.4, 14.4$  Hz, H-17), 2.25 (1H, m, H-15a), 2.18 (1H, m, H-4), 2.14 (3H, s, OAc-22), 2.06 (3H, s, OAc-16), 1.45 (1H, m, H-7), 1.30 (1H, dd,  $J = 14.2, 4.7$  Hz, H-15b), 1.18 (1H, d,  $J = 4.6$  Hz, H-19a), 1.16 (3H, s, H-18), 1.09 (3H, d,  $J = 7.0$  Hz, H-27), 1.08 (3H, d,  $J = 6.7$  Hz, H-28), 0.93 (3H, s, H-30), 0.89 (3H, d,  $J = 7.0$  Hz, H-21), 0.55 (1H, d,  $J = 4.6$  Hz, H-19);  $^{13}\text{C}$  NMR, see Table 2; HR-ESIMS  $m/z$  591.3291 (calcd for  $\text{C}_{34}\text{H}_{48}\text{O}_7\text{Na}$  591.3292).

**Preparation of 25 $\xi$ -Neoboutomellerone-26-al (13):** To a stirred solution of **1** (100 mg, 0.18 mmol) in  $\text{CH}_2\text{Cl}_2$  (3.5 mL) was added 350  $\mu\text{L}$  of pyridine (2 mL/mmol), and the mixture was cooled to 0  $^\circ\text{C}$ . The Dess-Martin reagent (1.76 mL, 0.528 mmol,  $c = 3$  mol/L, 3 equiv) was added dropwise, and the mixture was allowed to warm. After 3 h, the mixture was diluted with EtOAc, and a saturated solution of  $\text{Na}_2\text{S}_2\text{O}_3$  was added. The aqueous layer was extracted three times with EtOAc (10 mL), and the combined organic layers were washed successively with  $\text{NaHCO}_3$  (10 mL) and brine. The organic solution was dried over  $\text{MgSO}_4$  and concentrated under vacuum. The residue was purified by chromatography on silica gel (cyclohexane–EtOAc, 7:3 to 6:4) to give **13** (66 mg, 66%).  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ )  $\delta$  9.52 (1H, d,  $J = 0.6$  Hz, H-26), 6.94 (1H, d,  $J = 10.1$  Hz, H-1), 6.31 (1H, s, H-24a), 6.06 (1H, s, H-24), 5.90 (1H, d,  $J = 10.1$  Hz, H-2), 5.58 (1H, d,  $J = 2.1$  Hz, H-22), 5.10 (1H, dt,  $J = 4.4, 7.7$  Hz, H-16), 3.45 (1H, q,  $J = 7.2$  Hz, H-25), 2.64 (1H, m, H-20), 2.31 (1H, dd,  $J = 11.0, 7.6$  Hz, H-17), 2.09 (3H, s, OAc-22), 2.04 (3H, s, OAc-16), 1.38 (1H, dd,  $J = 13.7, 4.0$  Hz, H-15b), 1.25 (1H, d,  $J = 4.4$  Hz, H-19a), 1.18–1.22 (7H, m, H-7, -27, -18), 1.03 (3H, d,  $J = 6.7$  Hz, H-28), 0.96 (3H, s, H-30), 0.94 (1H, qd,  $J = 12.8, 3.7$  Hz, H-6b), 0.85 (3H, d,  $J = 7.0$  Hz, H-21), 0.58 (1H, d,  $J = 4.4$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 2; ESI-MS  $m/z$  567.3  $[\text{M} + \text{H}]^+$ .

**9,10-Di-epi-25 $\xi$ -neoboutomellerone (14):** yellow gum;  $[\alpha]_{\text{D}}^{20} -48$  (c 0.13,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 210 (3.73), 270 (3.93) nm; IR (film)  $\nu_{\text{max}}$  3417, 2937, 2875, 1734, 1666, 1373, 1233, 1022  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.39 (1H, d,  $J = 10.4$  Hz, H-1), 6.13 (1H, s, H-24a), 5.99 (1H, d,  $J = 10.4$  Hz, H-2), 5.88 (1H, d,  $J = 0.6$  Hz, H-24a), 5.58 (1H, d,  $J = 2.1$  Hz, H-22), 5.15 (1H, dt,  $J = 5.0, 7.7$  Hz, H-16), 3.60 (2H, t,  $J = 5.5$  Hz, 2 H-26), 2.88 (1H, sext,  $J = 6.4$  Hz, H-25), 2.62 (1H, m, H-20), 2.28 (1H, dd,  $J = 11.9, 7.3$  Hz, H-17), 2.26 (1H, m, H-15a), 2.20 (1H, m, H-4), 2.16 (3H, s, OAc-22), 2.10 (3H, s, OAc-16), 1.96 (1H, m, H-8), 1.85 (1H, dt,  $J = 5.0, 13.0$  Hz, H-12a), 1.6 (1H, dd,  $J = 13.0, 4.1$  Hz, H-12a), 1.28 (1H, d,  $J = 5.0$  Hz, H-19a), 1.14 (3H, s, H-18), 1.12 (3H, d,  $J = 6.1$  Hz, H-28), 1.10 (3H, s, H-27), 0.93 (3H, br s, H-30), 0.91 (3H, d,  $J = 7.0$  Hz, H-21), 0.88



(1H, d,  $J = 5.0$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 2; HR-ESIMS  $m/z$  591.3295 (calcd for  $\text{C}_{34}\text{H}_{48}\text{O}_5\text{Na}$ , 591.3292).

**9,10-Di-epi-22-de-O-acetyl-25 $\xi$ -neoboutomellerone (15):** yellow gum;  $[\alpha]_{\text{D}}^{20} -53$  (c 0.13,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 210 (4.12), 270 (4.20) nm; IR (film)  $\nu_{\text{max}}$  3421, 2937, 2877, 1732, 1666, 1609, 1453, 1376, 1240, 1024  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.39 (1H, d,  $J = 10.1$  Hz, H-1), 6.19 (1H, s, H-24a), 6.02 (1H, s, H-24a), 5.99 (1H, d,  $J = 10.1$  Hz, H-2), 5.39 (1H, dt,  $J = 5.0, 7.6$  Hz, H-16), 4.73 (1H, br s, H-22), 3.64 (1H, d,  $J = 5.8$  Hz, H-26), 3.54 (1H, br s, OH-22), 2.96 (1H, sext,  $J = 6.7$  Hz, H-25), 2.53 (1H, dd,  $J = 11.0, 7.6$  Hz, H-17), 2.48 (1H, m, H-20), 2.31 (1H, dd,  $J = 13.3, 7.8$  Hz, H-15), 2.11 (3H, s, OAc-16), 1.84 (1H, dt,  $J = 4.7, 13.4$  Hz, H-12), 1.60 (1H, ddd,  $J = 13.4, 4.0, 1.5$  Hz, H-12b), 1.33 (1H, m, H-15), 1.28 (1H, d,  $J = 4.9$  Hz, H-19a), 1.13 (6H, d,  $J = 7.0$  Hz, H-27, H-28); 1.12 (3H, s, H-18), 0.97 (3H, s, H-30), 0.88 (1H, d,  $J = 4.9$  Hz, H-19b), 0.67 (3H, d,  $J = 6.4$  Hz, H-21);  $^{13}\text{C}$  NMR, see Table 2; HR-ESIMS  $m/z$  549.3190 (calcd for  $\text{C}_{32}\text{H}_{46}\text{O}_6\text{Na}$ , 549.3187).

**26-Deoxyneoboutomellerone (16):** yellow gum;  $[\alpha]_{\text{D}}^{20} -102$  (c 0.08,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 220 (3.95), 264 (3.77) nm; IR (film)  $\nu_{\text{max}}$  3437, 2962, 2931, 2873, 1734, 1669, 1599, 1455, 1407, 1237  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.94 (1H, d,  $J = 10$  Hz, H-1), 5.94 (1H, s, H-24a), 5.90 (1H, d,  $J = 10.0$  Hz, H-2), 5.83 (1H, d,  $J = 1.2$  Hz, H-24a), 5.51 (1H, d,  $J = 2.4$  Hz, H-22), 5.09 (1H, dt,  $J = 4.3, 7.6$  Hz, H-16), 2.81 (1H, dsept,  $J = 0.9, 7.0$  Hz, H-25), 2.56 (1H, ddq,  $J = 11.0, 2.1, 7.0$  Hz, H-20), 2.29 (1H, dd,  $J = 11.0, 7.3$  Hz, H-17), 2.18 (2H, m, H-4, H-15a), 2.09 (3H, s, OAc-22), 2.02 (3H, s, OAc-16), 1.56 (1H, ddd,  $J = 7.0, 9.0, 15.0$  Hz, H-11b), 1.44 (1H, ddt,  $J = 7.0, 13.5, 4.0$  Hz, H-7a), 1.36 (1H, ddd,  $J = 14.0, 4.3, 0.9$  Hz, H-15b), 1.24 (1H, d,  $J = 4.6$  Hz, H-19a), 1.18 (3H, s, H-18), 1.07 (3H, d,  $J = 6.7$  Hz, H-26), 1.02 (3H, d,  $J = 7.0$  Hz, H-28), 1.00 (3H, d,  $J = 7.0$  Hz, H-27), 0.95 (3H, s, H-30), 0.85 (3H, d,  $J = 7.0$  Hz, H-21), 0.58 (1H, d,  $J = 4.6$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 2; HR-ESIMS  $m/z$  575.3341 (calcd for  $\text{C}_{34}\text{H}_{48}\text{O}_6\text{Na}$ , 575.3343).

**22-De-O-acetyl-26-deoxyneoboutomellerone (17):** yellow gum;  $[\alpha]_{\text{D}}^{20} -91$  (c 0.12,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 220 (3.87), 266 (3.86) nm; IR (film)  $\nu_{\text{max}}$  3457, 2933, 2931, 2873, 1734, 1669, 1456, 1375, 1237  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.93 (1H, d,  $J = 10.0$  Hz, H-1), 6.02 (1H, s, H-24a), 5.93 (1H, d,  $J = 1.2$  Hz, H-24a), 5.89 (1H, d,  $J = 10.0$  Hz, H-2), 5.20 (1H, dt,  $J = 4.4, 7.6$  Hz, H-16), 4.71 (1H, d,  $J = 5.8$  Hz, H-22), 3.54 (1H, d,  $J = 5.8$  Hz, OH-22), 2.85 (1H, dsept,  $J = 0.9, 6.9$  Hz, H-25), 2.42 (2H, m, H-17, H-20), 2.22 (1H, dd,  $J = 13.9, 7.8$  Hz, H-15a), 2.17 (1H, dq,  $J = 12.7, 6.9$  Hz, H-4), 2.02 (3H, s, OAc-16), 1.54 (1H, ddd,  $J = 7.0, 8.5, 15.0$  Hz, H-11), 1.45 (1H, ddt,  $J = 7.0, 14.0, 4.0$  Hz, H-7a), 1.37 (1H, ddd,  $J = 13.9, 4.4, 1.0$  Hz, H-15), 1.23 (1H, d,  $J = 4.3$  Hz, H-19a), 1.17 (3H, s, H-18), 1.09 (3H, d,  $J = 7.0$  Hz, H-26), 1.03 (3H, d,  $J = 6.7$  Hz, H-28), 1.02 (3H, d,  $J = 6.7$  Hz, H-27), 0.96 (3H, d,  $J = 0.6$  Hz, H-30), 0.64 (3H, d,  $J = 6.4$  Hz, H-21), 0.57 (1H, d,  $J = 4.3$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 3; HR-ESIMS  $m/z$  533.3234 (calcd for  $\text{C}_{32}\text{H}_{46}\text{O}_5\text{Na}$ , 533.3237).

**6 $\beta$ -Hydroxy-26-deoxyneoboutomellerone (18):** yellow gum;  $[\alpha]_{\text{D}}^{20} -61$  (c 0.12,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 220 (3.83), 266 (3.74) nm; IR (film)  $\nu_{\text{max}}$  3414, 2965, 2939, 2876, 1737, 1668, 1600, 1376, 1232  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.84 (1H, d,  $J = 9.9$  Hz, H-1), 5.95 (1H, s, H-24a), 5.84 (1H, d,  $J = 1.2$  Hz, H-24a), 5.82 (1H, d,  $J = 9.9$  Hz, H-2), 5.53 (1H, d,  $J = 2.1$  Hz, H-22), 5.10 (1H, dt,  $J = 4.3, 7.6$  Hz, H-16), 4.05 (1H, br s, H-6), 2.81 (1H, dsept,  $J = 1.0, 6.7$  Hz, H-25), 2.61 (1H, d,  $J = 3.4$  Hz, OH-6), 2.57 (1H, ddq,  $J = 2.2, 10.0, 6.9$  Hz, H-20), 2.48 (1H, dq,  $J = 13.1, 6.7$  Hz, H-4), 2.32 (1H, dd,  $J = 11.0, 7.6$  Hz, H-17), 2.29 (1H, dd,  $J = 12.8, 4.9$  Hz, H-8a), 2.22 (1H, dd,  $J = 13.7, 7.9$  Hz, H-15a), 2.21 (1H, m, H-11), 2.10 (3H, s, OAc-22), 2.03 (3H, s, OAc-16), 1.81 (1H, d,  $J = 3.1$  Hz, H-19a), 1.75 (2H, m, 2 H-12), 1.52 (1H, dt,  $J = 13.4, 4.7$  Hz, H-7), 1.24 (3H, s, H-18), 1.14 (3H, d,  $J = 6.7$  Hz, H-28), 1.08 (3H, d,  $J = 6.7$  Hz, H-26), 1.01 (3H, d,  $J = 7.0$  Hz, H-27), 1.00 (3H, s, H-30), 0.86 (3H, d,  $J = 7.0$  Hz, H-21), 0.67 (1H, d,  $J = 3.4$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 3; HR-ESIMS  $m/z$  591.3287 (calcd for  $\text{C}_{34}\text{H}_{48}\text{O}_7\text{Na}$ , 591.3292).

**1,2-Dihydro-22-de-O-acetyl-26-deoxyneoboutomellerone (19):** yellow gum;  $[\alpha]_{\text{D}}^{20} +19$  (c 0.09,  $\text{CHCl}_3$ ); IR (film)  $\nu_{\text{max}}$  3401, 2930, 2871, 1734, 1707, 1453, 1377, 1240  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)

$\delta$  6.03 (1H, s, H-24a), 5.94 (1H, d,  $J = 1.1$  Hz, H-24a), 5.20 (1H, td,  $J = 4.2, 7.7$  Hz, H-16), 4.70 (1H, d,  $J = 1.4$  Hz, H-22), 2.86 (1H, dsept,  $J = 0.9, 7.0$  Hz, H-25), 2.03 (3H, s, OAc-16), 1.86–1.78 (2H, m, H-1, H-12), 1.22 (3H, s, H-18), 1.10 (3H, d,  $J = 6.7$  Hz, H-27), 1.02 (3H, d,  $J = 6.7$  Hz, H-26), 0.97 (1H, d,  $J = 0.6$  Hz, H-30), 0.91 (3H, d,  $J = 6.8$  Hz, H-28), 0.76 (1H, dq,  $J = 2.3, 12.5$  Hz, H-6b), 0.64 (1H, m, H-19a), 0.64 (3H, d,  $J = 6.1$  Hz, H-21), 0.47 (1H, d,  $J = 4.0$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 3; HR-ESIMS  $m/z$  535.3393 (calcd for  $\text{C}_{32}\text{H}_{48}\text{O}_5\text{Na}$ , 535.3394).

**9,10-Di-epi-26-deoxyneoboutomellerone (20):** yellow gum;  $[\alpha]_{\text{D}}^{20} -98$  (c 0.11,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 212 (4.08), 270 (4.21) nm; IR (film)  $\nu_{\text{max}}$  3451, 2936, 2873, 2876, 1734, 1667, 1609, 1452, 1374, 1238  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.50 (1H, d,  $J = 10.2$  Hz, H-1), 5.95 (1H, s, H-24a), 5.88 (1H, d,  $J = 10.2$  Hz, H-2), 5.82 (1H, d,  $J = 1.2$  Hz, H-24a), 5.52 (1H, d,  $J = 2.0$  Hz, H-22), 5.14 (1H, dt,  $J = 4.5, 7.7$  Hz, H-16), 2.81 (1H, dsept,  $J = 1.0, 7.0$  Hz, H-25), 2.57 (1H, ddq,  $J = 10.9, 2.1, 6.9$  Hz, H-20), 2.29 (1H, dd,  $J = 10.8, 7.7$  Hz, H-17), 2.22 (2H, m, H-4, H-8a), 2.09 (3H, s, OAc-22), 2.04 (3H, s, OAc-16), 2.00 (1H, m, H-7), 1.83 (1H, dt,  $J = 4.4, 12.5, 5.0$  Hz, H-12a), 1.63 (1H, ddd,  $J = 2.0, 5.0, 13.0$  Hz, H-12b), 1.42 (1H, ddd,  $J = 2.2, 5.0, 14.0$  Hz, H-11b), 1.36 (1H, dd,  $J = 4.7, 14.0$  Hz, H-15b), 1.30 (1H, d,  $J = 4.7$  Hz, H-19a), 1.13 (3H, s, H-18), 1.07 (3H, d,  $J = 7.0$  Hz, H-26), 1.04 (3H, d,  $J = 6.6$  Hz, H-28), 1.00 (3H, d,  $J = 6.9$  Hz, H-27), 0.93 (3H, s, H-30), 0.92 (1H, m, H-19), 0.86 (3H, d,  $J = 6.9$  Hz, H-21);  $^{13}\text{C}$  NMR, see Table 3; HR-ESIMS  $m/z$  575.3338 (calcd for  $\text{C}_{34}\text{H}_{48}\text{O}_6\text{Na}$ , 575.3343).

**24a-Nor-24,25-didehydro-26-deoxyneoboutomellerone (21):** yellow gum;  $[\alpha]_{\text{D}}^{20} -73$  (c 0.03,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 242 (3.96) nm; IR (film)  $\nu_{\text{max}}$  3433, 2932, 2931, 2874, 1735, 1671, 1615, 1378, 1234  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.94 (1H, d,  $J = 10.1$  Hz, H-1), 6.14 (1H, sept,  $J = 1.3$  Hz, H-24), 5.90 (1H, d,  $J = 10.1$  Hz, H-2), 5.10 (1H, dt,  $J = 4.6, 7.8$  Hz, H-16), 4.85 (1H, d,  $J = 1.8$  Hz, H-22), 2.59 (1H, ddq,  $J = 10.9, 1.5, 7.0$  Hz, H-20), 2.12 (3H, d,  $J = 1.2$  Hz, H-27), 2.09 (3H, s, OAc-22), 2.08 (3H, s, OAc-16), 1.98 (3H, m, H-26), 1.26 (1H, d,  $J = 4.3$  Hz, H-19a), 1.20 (3H, s, H-18), 1.02 (3H, d,  $J = 6.7$  Hz, H-28), 0.93 (3H, d,  $J = 0.9$  Hz, H-30), 0.85 (3H, d,  $J = 7.0$  Hz, H-21), 0.56 (1H, d,  $J = 4.3$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 1; HR-ESIMS  $m/z$  561.3182 (calcd for  $\text{C}_{33}\text{H}_{46}\text{NaO}_6$ , 561.3187).

**22-De-O-acetyl-24a-nor-24,25-didehydro-26-deoxyneoboutomellerone (22):** yellow gum;  $[\alpha]_{\text{D}}^{20} -80$  (c 0.04,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 242 (3.95) nm; IR (film)  $\nu_{\text{max}}$  3464, 2933, 2874, 1732, 1671, 1618, 1449, 1378, 1237  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.94 (1H, d,  $J = 10$  Hz, H-1), 6.17 (1H, sept,  $J = 1.3$  Hz, H-24), 5.89 (1H, d,  $J = 10.0$  Hz, H-2), 5.19 (1H, dt,  $J = 4.7, 7.9$  Hz, H-16), 3.99 (1H, dd,  $J = 4.9, 1.2$  Hz, H-22), 3.54 (1H, d,  $J = 5.2$  Hz, OH-22), 2.47 (1H, ddq,  $J = 11.3, 1.8, 7.0$  Hz, H-20), 2.35 (1H, dd,  $J = 11.0, 7.6$  Hz, H-17), 2.18 (1H, m, H-4), 2.17 (3H, d,  $J = 1.2$  Hz, H-27), 2.05 (3H, s, OAc-16), 1.94 (3H, m, H-26), 1.56 (1H, ddd,  $J = 7.5, 8.5, 14.0$  Hz, H-11), 1.47 (1H, m, H-7), 1.39 (1H, dd,  $J = 13.6, 5.0$  Hz, H-15), 1.25 (1H, d,  $J = 4.6$  Hz, H-19a), 1.20 (3H, s, H-18), 1.02 (3H, d,  $J = 6.7$  Hz, H-28), 0.95 (3H, d,  $J = 0.6$  Hz, H-30), 0.65 (3H, d,  $J = 6.7$  Hz, H-21), 0.56 (1H, d,  $J = 4.6$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 3; HR-ESIMS  $m/z$  519.3076 (calcd for  $\text{C}_{31}\text{H}_{44}\text{O}_5\text{Na}$ , 519.3081).

**9,10-Di-epi-24a-nor-24,25-didehydro-26-deoxyneoboutomellerone (23):** yellow gum;  $[\alpha]_{\text{D}}^{20} -55$  (c 0.07,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 210 (3.77), 244 (3.98) nm; IR (film)  $\nu_{\text{max}}$  3409, 2936, 2874, 1734, 1669, 1612, 1447, 1374, 1235  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  6.50 (1H, d,  $J = 10.1$  Hz, H-1), 6.15 (1H, sept,  $J = 1.2$  Hz, H-24), 5.88 (1H, d,  $J = 10.1$  Hz, H-2), 5.16 (1H, dt,  $J = 4.8, 7.7$  Hz, H-16), 4.86 (1H, d,  $J = 1.7$  Hz, H-22), 2.62 (1H, ddq,  $J = 11.0, 1.5, 6.9$  Hz, H-20), 2.12 (3H, d,  $J = 1.1$  Hz, H-27), 2.10 (6H, 2 s, OAc-16, OAc-22), 1.44–1.35 (2H, m, H-11, H-15), 1.30 (1H, d,  $J = 4.9$  Hz, H-19a), 1.15 (3H, s, H-18), 1.05 (3H, d,  $J = 6.8$  Hz, H-28), 0.93 (1H, m, H-19b), 0.91 (3H, d,  $J = 1.0$  Hz, H-30), 0.86 (3H, d,  $J = 7.0$  Hz, H-21);  $^{13}\text{C}$  NMR, see Table 3; HR-ESIMS  $m/z$  561.3187 (calcd for  $\text{C}_{33}\text{H}_{46}\text{O}_6\text{Na}$ , 561.3187).

**23,24,24a,25,26,27-Hexa-nor-neoboutomellerone-22-al (24):** yellow gum;  $[\alpha]_{\text{D}}^{20} -74$  (c 0.11,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 210



(3.51), 266 (3.83) nm; IR (film)  $\nu_{\max}$  3640, 2938, 2874, 1732, 1669, 1603, 1455, 1375, 1238  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ , 500 MHz)  $\delta$  9.58 (1H, d,  $J = 1.8$  Hz, H-22), 6.95 (1H, d,  $J = 10.0$  Hz, H-1), 5.90 (1H, d,  $J = 10.0$  Hz, H-2), 5.24 (1H, dt,  $J = 5.5, 8.2$  Hz, H-16), 2.86 (1H, ddq,  $J = 11.0, 2.0, 7.3$  Hz, H-20), 2.39 (1H, dd,  $J = 11.0, 8.2$  Hz, H-17), 2.16 (1H, m, H-4), 1.89 (3H, s, OAc-16), 1.47 (1H, m, H-7), 1.33 (1H, ddq,  $J = 13.4, 5.6, 1.1$  Hz, H-15), 1.27 (1H, d,  $J = 4.3$  Hz, H-19a), 1.17 (3H, s, H-18), 1.09 (3H, d,  $J = 7.3$  Hz, H-21), 1.02 (3H, d,  $J = 6.7$  Hz, H-28), 0.96 (3H, d,  $J = 0.9$  Hz, H-30), 0.55 (1H, d,  $J = 4.3$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 3; HR-ESIMS  $m/z$  435.2503 (calcd for  $\text{C}_{26}\text{H}_{36}\text{O}_4\text{Na}$ , 435.2506).

**23,24,24a,25,26,27-Hexa-nor-neoboutomelleron-22-oic acid (25):** yellow gum;  $[\alpha]_{\text{D}}^{20} -57$  (c 0.06,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 208 (3.52), 266 (3.74) nm; IR (film)  $\nu_{\max}$  3406, 2930, 2875, 1725, 1666, 1456, 1376, 1247  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.86 (1H, d,  $J = 10.0$  Hz, H-1), 6.01 (1H, d,  $J = 10.0$  Hz, H-2), 5.46 (1H, br q,  $J = 7.3$  Hz, H-16), 2.73 (1H, m, H-20), 2.42 (1H, dd,  $J = 10.2, 9.1$  Hz, H-17), 2.20 (1H, dq,  $J = 12.0, 6.9$  Hz, H-4), 2.00 (3H, s, OAc-16), 1.75 (1H, m, H-12), 1.35 (1H, m, H-15), 1.23 (1H, d,  $J = 4.5$  Hz, H-19a), 1.21 (3H, d,  $J = 7.0$  Hz, H-21), 1.16 (3H, s, H-18), 1.10 (3H, d,  $J = 6.8$  Hz, H-28), 0.96 (3H, s, H-30), 0.55 (1H, d,  $J = 4.5$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 4; HR-ESIMS  $m/z$  451.2428 (calcd for  $\text{C}_{26}\text{H}_{36}\text{O}_5\text{Na}$ , 451.2455).

**16-Acetyl-3 $\beta$ ,26-dihydroxy-24-methyl-25 $\xi$ -cycloart-24(24a)-en-23-on-29-oic acid (26):** yellow gum;  $[\alpha]_{\text{D}}^{20} -7$  (c 0.04,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 220 (3.57) nm; IR (film)  $\nu_{\max}$  3310, 2933, 2871, 1732, 1669, 1555, 1378, 1247, 1028  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.03 (1H, s, H-24a), 5.75 (1H, s, H-24a), 5.26 (1H, dt,  $J = 4.9, 7.8$  Hz, H-16), 4.07 (1H, br d,  $J = 7.9$  Hz, H-3), 3.55 (1H, dd,  $J = 10.6, 5.2$  Hz, H-26a), 3.49 (1H, dd,  $J = 10.6, 7.0$  Hz, H-26b), 2.95 (1H, sext,  $J = 6.5$  Hz, H-25), 2.52 (3H, m, H-20, 2 H-22), 2.11 (1H, dd,  $J = 13.7, 8.2$  Hz, H-15), 1.95 (3H, s, OAc-16), 1.89 (1H, dd,  $J = 9.9, 8.1$  Hz, H-17), 1.80 (1H, m, H-2), 1.17 (3H, s, H-18), 1.13 (3H, br.s, H-28), 1.05 (3H, d,  $J = 7.0$  Hz, H-27), 0.91 (3H, s, H-30), 0.88 (3H, d,  $J = 5.8$  Hz, H-21), 0.62 (1H, d,  $J = 4.0$  Hz, H-19a), 0.40 (1H, d,  $J = 4.0$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 4; HRESIMS  $m/z$  581.3443 (calcd for  $\text{C}_{33}\text{H}_{50}\text{O}_7\text{Na}$ , 581.3449).

**16-Acetyl-3 $\beta$ ,22 $\beta$ -dihydroxy-24-methylcycloart-24(24a)-en-23-on-29-oic acid (27):** yellow gum;  $[\alpha]_{\text{D}}^{20} 0$  (c 0.03,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 216 (3.58) nm; IR (film)  $\nu_{\max}$  3302, 2929, 2864, 1732, 1671, 1542, 1454, 1385  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (methanol- $d_4$ , 500 MHz)  $\delta$  5.99 (1H, s, H-24a), 5.89 (1H, d,  $J = 1.2$  Hz, H-24a), 5.27 (1H, dt,  $J = 4.0, 7.3$  Hz, H-16), 4.71 (1H, d,  $J = 1.5$  Hz, H-22), 4.03 (1H, dd,  $J = 11.3, 4.6$  Hz, H-3), 2.87 (1H, dsept,  $J = 1, 6.7$  Hz, H-25), 2.24 (1H, dd,  $J = 14.0, 7.9$  Hz, H-15a), 2.06 (3H, s, OAc-16), 1.94 (1H, dd,  $J = 12.2, 3.4$  Hz, H-5), 1.72 (1H, m, H-2), 1.19 (3H, s, H-18), 1.11 (3H, d,  $J = 7.0$  Hz, H-27), 1.07 (3H, s, H-28), 1.03 (3H, d,  $J = 7.0$  Hz, H-26), 0.99 (3H, s, H-30), 0.69 (3H, d,  $J = 6.1$  Hz, H-21), 0.63 (1H, d,  $J = 4.1$  Hz, H-19a), 0.42 (1H, d,  $J = 4.1$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 4; HR-ESIMS  $m/z$  581.3455 (calcd for  $\text{C}_{33}\text{H}_{50}\text{O}_7\text{Na}$ , 581.3449).

**3 $\beta$ ,16 $\beta$ ,22 $\beta$ -Trihydroxy-24-methyl-(16,23:23,26)-diepoxycycloart-24(24a)-en-29-oic acid (28):** yellow gum;  $[\alpha]_{\text{D}}^{20} -38$  (c 0.05,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 208 (3.41), 270 (2.74) nm; IR (film)  $\nu_{\max}$  3300, 2929, 2864, 1650, 1541, 1385  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (methanol- $d_4$ , 500 MHz)  $\delta$  5.24 (1H, d,  $J = 3.1$  Hz, H-24a), 4.92 (1H, d,  $J = 3.1$  Hz, H-24a), 4.27 (1H, dt,  $J = 8.6, 7.1$  Hz, H-16), 4.03 (1H, dd,  $J = 11.3, 4.6$  Hz, H-3), 3.94 (1H, t,  $J = 7.8$  Hz, H-27a), 3.30 (1H, t,  $J = 7.8$  Hz, H-27b), 2.75 (1H, m, H-25), 2.07 (1H, ddd,  $J = 15.3, 10.5, 5.3$  Hz, H-7a), 1.95 (1H, dd,  $J = 12.4, 4.1$  Hz, H-5), 1.90 (1H, m, H-20), 1.84 (1H, dd,  $J = 12.8, 7.9$  Hz, H-15a), 1.78 (1H, dd,  $J = 13.7, 2.7$  Hz, H-22a), 1.74 (1H, m, H-2a), 1.49 (1H, t,  $J = 13.7$  Hz, H-22b), 1.41 (1H, br dd,  $J = 12.5, 6.7$  Hz, H-15b), 1.18 (3H, s, H-18), 1.08 (3H, s, H-28), 1.07 (3H, d,  $J = 6.8$  Hz, H-26), 0.93 (3H, d,  $J = 7.0$  Hz, H-21), 0.92 (3H, s, H-30), 0.68 (1H, d,  $J = 4.0$  Hz, H-19a), 0.41 (1H, d,  $J = 4.0$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 4; HR-ESIMS  $m/z$  521.3230 (calcd for  $\text{C}_{31}\text{H}_{46}\text{O}_8\text{Na}$ , 521.3237).

**3 $\beta$ -Hydroxy-24-methylcycloart-24(24a)-en-23-on-29-oic acid (29):** yellow gum;  $[\alpha]_{\text{D}}^{20} -34$  (c 0.06,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 220 (3.78) nm; IR (film)  $\nu_{\max}$  3380, 2930, 2869, 1678, 1552, 1406, 1374  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (methanol- $d_4$ , 500 MHz)  $\delta$  6.05 (1H, s, H-24a), 5.75 (1H, d,  $J = 0.9$  Hz, H-24a), 4.02 (1H, dd,  $J = 11.4, 4.4$  Hz,

H-3), 2.87 (1H, dsept,  $J = 1.0, 6.8$  Hz, H-25), 2.72 (1H, dd,  $J = 15.6, 3.1$  Hz, H-22a), 2.48 (1H, dd,  $J = 15.6, 10.1$  Hz, H-22b), 1.08 (3H, s, H-28), 1.04 (3H, s, H-18), 1.02 (3H, d,  $J = 6.7$  Hz, H-27), 1.01 (3H, d,  $J = 7.0$  Hz, H-26), 0.95 (3H, s, H-30), 0.84 (3H, d,  $J = 6.4$  Hz, H-21), 0.62 (1H, d,  $J = 4.2$  Hz, H-19a), 0.41 (1H, d,  $J = 4.2$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 4; HR-ESIMS  $m/z$  507.3441 (calcd for  $\text{C}_{31}\text{H}_{48}\text{O}_4\text{Na}$ , 507.3445).

**3 $\beta$ -Hydroxy-29-O-( $\alpha$ -L-rhamnopyranosyl)cycloart-24-en-23-on-29-oic acid (30):** yellow gum;  $[\alpha]_{\text{D}}^{20} -22$  (c 0.17,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 238 (4.27), 300 (3.09) nm; IR (film)  $\nu_{\max}$  3291, 2932, 2867, 1723, 1677, 1607, 1445, 1378, 1311  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (methanol- $d_4$ , 500 MHz)  $\delta$  6.16 (1H, sept,  $J = 1.2$  Hz, H-24), 5.99 (1H, d,  $J = 1.8$  Hz, H-1'), 4.03 (1H, dd,  $J = 11.3, 4.3$  Hz, H-3), 3.86 (1H, dd,  $J = 3.4, 1.8$  Hz, H-2'), 3.69 (1H, dd,  $J = 9.5, 3.4$  Hz, H-3'), 3.61 (1H, dq,  $J = 9.5, 6.2$  Hz, H-5'), 3.44 (1H, t,  $J = 9.5$  Hz, H-4'), 2.52 (1H, dd,  $J = 15.0, 3.1$  Hz, H-22a), 2.11 (3H, d,  $J = 0.9$  Hz, H-27), 1.99 (1H, dd,  $J = 12.1, 3.8$  Hz, H-5), 1.90 (3H, d,  $J = 0.9$  Hz, H-26), 1.22 (3H, d,  $J = 6.1$  Hz, H-6'), 1.13 (3H, s, H-28), 1.04 (3H, s, H-18), 0.94 (3H, s, H-30), 0.86 (3H, d,  $J = 6.4$  Hz, H-21), 0.64 (1H, d,  $J = 4.1$  Hz, H-19a), 0.44 (1H, d,  $J = 4.1$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 4; HR-ESIMS  $m/z$  639.3856 (calcd for  $\text{C}_{36}\text{H}_{56}\text{O}_8\text{Na}$ , 639.3867).

**3 $\beta$ -Hydroxy-29-O-( $\alpha$ -L-rhamnopyranosyl)-24-methylcycloart-24(24a)-en-23-on-29-oic acid (31):** yellow gum;  $[\alpha]_{\text{D}}^{20} -16$  (c 0.13,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 220 (3.95) nm; IR (film)  $\nu_{\max}$  3291, 2935, 2865, 1725, 1659, 1462, 1376, 1248, 1147, 1105  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (methanol- $d_4$ , 500 MHz)  $\delta$  6.05 (1H, s, H-24a), 5.99 (1H, d,  $J = 1.8$  Hz, H-1'), 5.76 (1H, d,  $J = 1.2$  Hz, H-24a), 4.03 (1H, dd,  $J = 4.3, 11$  Hz, H-3), 3.86 (1H, dd,  $J = 3.5, 1.8$  Hz, H-2'), 3.70 (1H, dd,  $J = 9.5, 3.5$  Hz, H-3'), 3.61 (1H, dq,  $J = 9.5, 6.2$  Hz, H-5'), 3.45 (1H, t,  $J = 9.5$  Hz, H-4'), 2.87 (1H, sept,  $J = 6.7$  Hz, H-25), 2.72 (1H, dd,  $J = 15.6, 2.7$  Hz, H-22a), 2.48 (1H, dd,  $J = 15.6, 9.8$  Hz, H-22b), 1.22 (3H, d,  $J = 6.2$  Hz, H-6'), 1.12 (3H, s, H-28), 1.04 (3H, s, H-18), 1.02 (3H, d,  $J = 7.0$  Hz, H-27), 1.01 (3H, d,  $J = 7.0$  Hz, H-26), 0.95 (3H, s, H-30), 0.84 (3H, d,  $J = 6.4$  Hz, H-21), 0.64 (1H, d,  $J = 4.1$  Hz, H-19a), 0.44 (1H, d,  $J = 4.1$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 4; HR-ESIMS  $m/z$  653.4014 (calcd for  $\text{C}_{37}\text{H}_{58}\text{O}_8\text{Na}$ , 653.4024).

**16,22-Diacetyl-2,26-dihydroxy-29-nor-24-methyl-19(9 $\rightarrow$ 1)-abeo-cycloart-9(11),24(24a)-dien-3,23-dione (32):** yellow gum;  $[\alpha]_{\text{D}}^{20} +77$  (c 0.13,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 210 (3.97), 268 (3.17) nm; IR (film)  $\nu_{\max}$  3394, 2926, 2854, 1734, 1694, 1566, 1376, 1229, 1021  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.12 (1H, s, H-24a), 5.87 (1H, d,  $J = 0.6$  Hz, H-24a), 5.56 (1H, d,  $J = 2.1$  Hz, H-22), 5.20 (1H, d,  $J = 6.1$  Hz, H-11), 5.09 (1H, dt,  $J = 4.4, 7.6$  Hz, H-16), 4.16 (1H, d,  $J = 2.1$  Hz, H-2), 3.60 (3H, m, OH-2, 2 H-26), 2.88 (1H, sext,  $J = 6.5$  Hz, H-25), 2.56 (1H, m, H-20), 2.36 (1H, dd,  $J = 14.0, 7.9$  Hz, H-15a), 2.27 (1H, dd,  $J = 11.1, 7.5$  Hz, H-17), 2.15 (3H, s, OAc-22), 2.07 (3H, s, OAc-16), 1.88 (1H, dd,  $J = 9.9, 4.7$  Hz, H-1), 1.37 (1H, dd,  $J = 14.2, 4.1$  Hz, H-15b), 1.11 (3H, d,  $J = 7.3$  Hz, H-27), 1.05 (3H, d,  $J = 6.4$  Hz, H-28), 0.94 (1H, bt,  $J = 4.7$  Hz, H-19a), 0.88 (3H, d,  $J = 7.0$  Hz, H-21), 0.81 (3H, s, H-30), 0.79 (3H, s, H-18), 0.41 (1H, dd,  $J = 9.9, 4.7$  Hz, H-19b);  $^{13}\text{C}$  NMR, see Table 4; HR-ESIMS  $m/z$  607.3245 (calcd for  $\text{C}_{34}\text{H}_{48}\text{O}_8\text{Na}$ , 607.3241).

**Proteasome Assay.** Experimental procedures for the assay are detailed in ref 3.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

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