

# CONSTITUENTS OF HIGH ALTITUDE HIMALAYAN HERBS, PART III.<sup>1</sup> NEW ALKANE HYDROXYKETONES FROM *TANACETUM NUBIGENUM*

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**ABSTRACT.**—*Tanacetum nubigenum* (Compositae) is a high altitude herb of Kumaon Himalayan Glaciers. Traditionally it is best used as an herbal perfume in the region. Two new alkane hydroxyketones: 22-hydroxyoctacosan-25-one, 24-hydroxytricontan-27-one along with tetracosanoic acid, hexacosanoic acid,  $\beta$ -sitosterol and sitosterol- $\beta$ -D-glucoside have been isolated from its petroleum ether extract and identified by chemical and ir, <sup>1</sup>H nmr and ms methods.

*Tanacetum nubigenum* Wallich ex DC. (Compositae), a small herb growing at an altitude above 1400 ft in the Kumaon Himalayan region of India, is pleasantly aromatic. The herb grows as the snow melts during June–July in the glacier region and lasts only until the next snow-fall in September–October. The plant is used by the local inhabitants as an herbal perfume. In a research program (1,2) to investigate high altitude herbs for active constituents, this plant was chemically investigated; two new aliphatic hydroxy ketones, 22-hydroxyoctacosan-25-one and 24-hydroxytricontan-27-one were isolated and identified along with other known compounds.

Compound **1**, mp 70–71°, gave a

positive 2,4-dinitrophenyl hydrazine test and showed prominent ir bands at  $\text{cm}^{-1}$  3440 (OH), 2920, 2860, 1705 (C=O), and 715 indicative of a saturated long chain aliphatic hydroxy ketone. The high resolution mass spectrum displayed an  $[M]^+$  at  $m/z$  424 corresponding to the structural formula  $\text{C}_{28}\text{H}_{56}\text{O}_2$ . The appearance of two series of peaks,  $m/z$  41, 55, 69 and 43, 57, 71, 85, with fragments differing by 14 mass units suggested the presence of a long straight methylene chain (3). This was further supported by the absence of an  $[M - 15]^+$  peak (4). In addition, the presence of an  $[M + 1]^+$  peak is characteristic of an unsymmetrical ketone (3). The position of the carbonyl group at C-

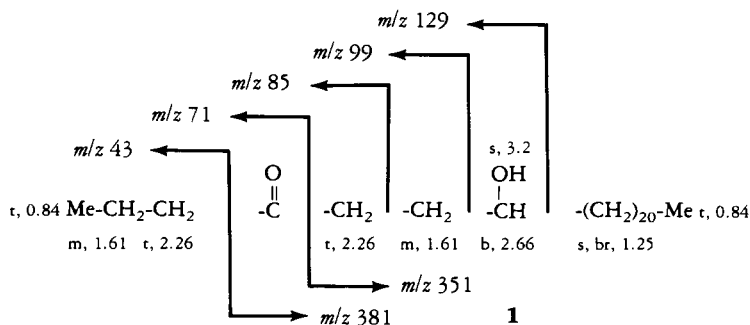


FIGURE 1. Ms and <sup>1</sup>H-nmr values (in  $\delta$  scale) for compound **1**: s, singlet; t, triplet; m, multiplet; b, broad; br, branched; Me, methyl group.

<sup>1</sup>For Part II see K.S. Khetwal and R.S. Bisht, *Phytochemistry*, **27**, 1910 (1988).

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25 was indicated by the  $\alpha$  fission fragments at  $m/z$  43 (base peak), 381, 71, and 353 and  $\beta$  fission fragments involving McLafferty rearrangement at  $m/z$  100 and 382 (5). This double rearrange-

ment, frequently observed in long chain aliphatic ketones, was further supported by an ion at  $m/z$  58. The hydroxyl group was located at C-22 by the significant  $\alpha$  fission ions at  $m/z$  99, 325, and 129.  $^1\text{H-Nmr}$  spectra gave signals for two terminal methyl groups at  $\delta$  0.84 (6H, t,  $\text{H}_3$ -1,  $\text{H}_3$ -30,  $J = 6.5$  Hz) and 20 methylene groups at 1.25 [44H, s br,  $(\text{CH}_2)_{20}$ ]. A triplet at  $\delta$  2.26 (4H, t,  $\text{H}_2$ -24,  $\text{H}_2$ -26), was indicative of two methylene groups  $\alpha$  to a carbonyl function. The signal at  $\delta$  1.61 (4H, t,  $\text{H}_2$ -23,  $\text{H}_2$ -27) was assigned to the methylene groups  $\beta$  to the C-25 carbonyl. The CH-OH and CH-OH protons were located at  $\delta$  3.2 and 2.66, respectively. These data were in accord with 22-hydroxyoctacosan-25-one [1].

Compound 2, mp 73–74°, crystallized from MeOH, had ir bands at 3440 (OH), 2920, 2860, 1705 ( $\text{C}=\text{O}$ ), 1460, 735, and 715. It gave a positive DNPH test, and the long chain unsymmetric nature of the ketone was indicated by the presence of  $[\text{M} + 1]^+$  and the absence of  $[\text{M} - 15]^+$  fragments in the mass spectrum (3,4). The long chain nature of the compound was also supported by two series of fragments with a uniform loss of 14 mass units (3). A parent ion at  $m/z$  452 ( $\text{C}_{30}\text{H}_{60}\text{O}_2$ ) was observed, along with fission ions at 72, 99, 325, and 129, indicating the position of OH at C-24. The base peak at  $[\text{M}]^+ 43$ ,  $\alpha$  fission fragments at 409, 71, and 381, and  $\beta$  fission fragments at  $m/z$  100 and 410 in-

volving McLafferty rearrangement indicated the presence of a carbonyl function at C-27 (5).  $^1\text{H-nmr}$  spectra gave signals for terminal methyl groups at  $\delta$  0.84 (6H, t,  $\text{H}_3$ -1,  $\text{H}_3$ -30,  $J = 6.5$  Hz) and 22 methylene groups at 1.25 [44H, s br,  $(\text{CH}_2)_{22}$ ]. The four-proton triplet at 2.26 ( $J = 6.0$  Hz) was indicative of two methylene groups adjacent to the carbonyl group and a multiplet at  $\delta$  3.26 for the CH-OH proton. The signal at  $\delta$  1.61 (4H, t,  $\text{H}_2$ -25,  $\text{H}_2$ -29) was due to two methylene groups  $\beta$  to a carbonyl function, whereas the CH-OH proton was observed at  $\delta$  2.66. These data are consistent with 24-hydroxytricontan-27-one [2].

Compounds 1 and 2 are new, and, to the best of our knowledge, have not previously been found in nature. Additional compounds isolated were identified as tetracosanoic and hexacosanoic acids and  $\beta$ -sitosterol and sitosterol- $\beta$ -D-glucoside.

## EXPERIMENTAL

### GENERAL EXPERIMENTAL PROCEDURES.—

Melting points are uncorrected. Ir spectra (Perkin Elmer Model-298) were recorded in KBr;  $^1\text{H-nmr}$  spectra (100 MHz Bruker) were determined in  $\text{CDCl}_3$  using TMS as internal standard. Ms (70 eV Jeol-JMS-300) was based on direct insertion. Separation by cc and tlc utilized Si gel G (60–120 mesh). A Waters Associates hplc system fitted with variable wavelength (190–750 nm) uv and ri detectors was used for analytical purposes. Tlc zones were visualized either by exposure to  $\text{I}_2$  vapor or 50%  $\text{H}_2\text{SO}_4$ , or under long range uv light (365 nm).

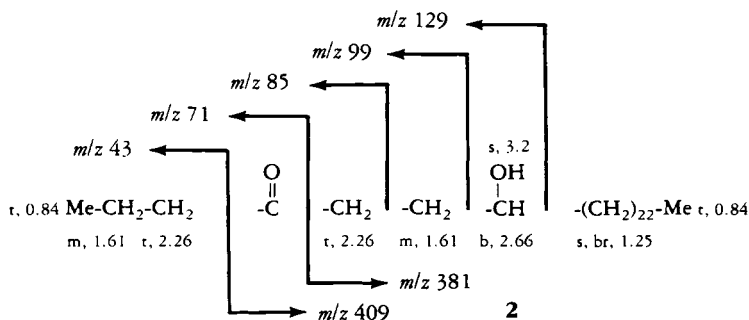


FIGURE 2. Ms and  $^1\text{H}$  nmr values (in  $\delta$  scale) for compound 2: s, singlet; t, triplet; m, multiplet; b, broad; br, branched; Me, methyl group.

**PLANT MATERIAL.**—The plant material was collected from Kumaon Himalayan glaciers in Uttar Pradesh, India at an altitude of 14,000 ft and identified in the department of Botany, Kumaon University, Nainital, where the voucher specimen is stored.

**EXTRACTION AND ISOLATION OF CONSTITUENTS.**—The whole plant was shade-dried, powdered, and Soxhlet-extracted with 90% EtOH. The EtOH extract was concentrated under vacuo and the residue further extracted with petroleum ether (60–80°). The petroleum ether extract was column chromatographed on Si gel G, eluting with petroleum ether (100%), petroleum ether-C<sub>6</sub>H<sub>6</sub> (99:1), petroleum ether-C<sub>6</sub>H<sub>6</sub> (98:2), petroleum ether-C<sub>6</sub>H<sub>6</sub> (95:5), petroleum ether-C<sub>6</sub>H<sub>6</sub> (50:50), and petroleum ether-C<sub>6</sub>H<sub>6</sub> (30:70). Compounds **1** and **2** were isolated and purified by repeated tlc and hplc methods.

**22-HYDROXYOCTACOSAN-25-ONE [1].**—Removal of the solvent petroleum ether-C<sub>6</sub>H<sub>6</sub> (99:1) afforded a residue (20 mg): mp 70–71° (MeOH); ir (KBr)  $\text{cm}^{-1}$  3440, 2920, 2860, 1705, 1466, 1175, 715; ms  $m/z$  [M]<sup>+</sup> 424 (C<sub>28</sub>H<sub>56</sub>O<sub>2</sub>), 410, 409, 396, 382, 381, 368, 354, 353, 340, 325, 311, 297, 283, 269, 241, 227, 213, 199, 185, 129, 111, 100, 99, 98, 97, 85, 73, 71, 58, 57, 43 (100%), 41; <sup>1</sup>H nmr (CDCl<sub>3</sub>) see Figure 1.

**24-HYDROXYTRICONTAN-27-ONE [2].**—Removal of the solvent petroleum ether-C<sub>6</sub>H<sub>6</sub> (99:1) afforded a residue (25 mg): mp 73–74° (MeOH); ir (KBr)  $\text{cm}^{-1}$  3440, 2920, 2860, 1705, 1466, 1175, 715; ms  $m/z$  [M]<sup>+</sup> 452 (C<sub>30</sub>H<sub>60</sub>O<sub>2</sub>), 424, 396, 382, 381, 368, 354, 353, 340, 325, 311, 297, 283, 269, 241, 227, 213, 199, 185, 129, 111, 100, 99, 98, 97, 85, 73, 71, 85, 57, 43 (100%), 41; <sup>1</sup>H nmr (CDCl<sub>3</sub>) see Figure 2.

**ADDITIONAL COMPOUNDS.**—The fractions

obtained from MeOH gave tetracosanoic acid (8 mg), mp 83.5° [lit. (5) mp 84.2°], identified by ir, co-tlc, co-hplc, and ms  $m/z$  [M]<sup>+</sup> 368 (C<sub>24</sub>H<sub>48</sub>O<sub>2</sub>). Further elution with petroleum ether-C<sub>6</sub>H<sub>6</sub> (95:5) gave hexacosanoic acid (10 mg), mp 86.5° (MeOH) [lit. (5) 87.7°], identified by ir, co-tlc, co-hplc, and ms  $m/z$  [M]<sup>+</sup> 396 (C<sub>26</sub>H<sub>52</sub>O<sub>2</sub>). Eluates from petroleum ether-C<sub>6</sub>H<sub>6</sub> (50:50) afforded sitosterol (80 mg), mp 136–137° [lit. (6) 136–137°], identified by mmp, co-tlc, co-hplc, ir, and <sup>1</sup>H nmr; ms  $m/z$  [M]<sup>+</sup> 414 (C<sub>29</sub>H<sub>50</sub>O). Fractions obtained from EtOAc-C<sub>6</sub>H<sub>6</sub> (30:70) afforded sitosterol-β-D-glucoside, (15 mg) mp 280–284° [lit. (6) 284–285°], identified by mmp, ir, co-tlc, co-hplc, ir, fdms [M + H]<sup>+</sup> 577 (C<sub>35</sub>H<sub>60</sub>O<sub>6</sub>), and hydrolytic degradation.

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