Phytochemistry, Vol. 25, No. 9, pp. 2201–2203, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

SAPONINS AND OTHER CONSTITUENTS FROM THE RHIZOMES OF *PANAX PSEUDO-GINSENG* SUBSP. *HIMALAICUS* VAR. *ANGUSTIFOLIUS**

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(*Revised received* 17 *January* 1986)

Key Word Index—*Panax pseudo-ginseng* subsp. *himalaicus* var. *angustifolius*; Araliaceae; rhizomes; ginsenosides Ro and Rb_1; chikusetsusaponin-IVa; 24-hydroxyhexatetracontanoic acid; 2-methylhexatetracont-1-en-3,21-diol; tritriacontanyl octacosanoate; tritriacontanol.

Abstract—Besides ginsenosides Ro and Rb_1, chikusetsusaponin-IVa and tritriacontanol, three new compounds have been isolated from the rhizomes of *Panax pseudo-ginseng* subsp. *himalaicus* var. *angustifolius* and characterized as 24-hydroxyhexatetracontanoic acid, 2-methylhexatetracont-1-en-3,21-diol and tritriacontanyl octacosanoate by physicochemical data.

INTRODUCTION

Panax pseudo-ginseng Wall. subsp. *himalaicus* Hara (Araliaceae) and its two varieties, var. *angustifolius* (Burk.) Li and var. *bipinnatifidus* (Seem.) Li are found in Singalila range of Darjeeling, West Bengal, India. Since ginseng has been attributed with a variety of medicinal properties and no work has been reported on Indian ginseng, a systematic chemical investigation was undertaken. In a previous communication, we have characterized several fatty acids and esters from the rhizomes of *P. pseudo-ginseng* subsp. *himalaicus* var. *angustifolius* [1]. Our continued interest in this plant has led to the isolation of three saponins and four aliphatic constituents which are reported herein.

RESULTS AND DISCUSSION

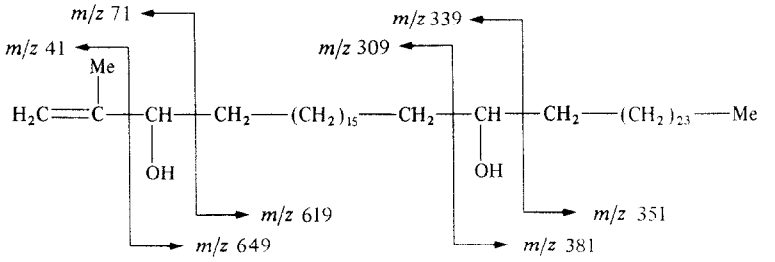
The 50% ethanol extract of the rhizomes was divided into *n*-hexane and *n*-butanol soluble fractions. Silica gel column chromatography of the *n*-butanol fraction furnished a mixture of saponins which was separated by preparative TLC to afford compounds **1**–**3**. Compound **1** was identified as ginsenoside-Rb_1 [2], compound **2** as chikusetsusaponin-IVa [3, 4] and compound **3** as ginsenoside-Ro [2] by comparison with authentic samples. Characterization of compounds **2** and **3** was verified by identification of their hydrolysis products as oleanolic acid, glucuronic acid and glucose.

Silica gel column chromatography of the *n*-hexane fraction furnished compounds **4**–**7**. The IR spectrum of compound **7**, mp 122°, showed bands for hydroxyl (3340 cm^{-1}), carboxyl (1715, 2500–3300 cm^{-1}) and a saturated straight chain (2920, 2850, 1465, 720 cm^{-1}). A molecular ion at *m/z* 692 in the mass spectrum of **7** suggested the molecular formula as $C_{46}H_{92}O_3$. The terminal position of the carboxyl group was evident from the significant ions at *m/z* 45, 60 and 632 $[M-60]^+$. The ion at *m/z* 60 was due to McLafferty rearrangement [5] involving β-fission of the carboxyl function. The attachment of the hydroxyl group at C-24 was shown by the prominent α-fission ions at *m/z* 383, 353, 339 and 309. The ^1H NMR spectrum of **7** displayed a triplet at δ2.25 due to a methylene group adjacent to a carboxyl group and a multiplet at δ3.65 for a –CH(OH)–proton. The above data were fully consistent with the structure of 24-hydroxyhexatetracontanoic acid.

Compound **6** (amorphous) had ν_{max} 3330 and 3220 (OH groups), 1620, 1400, 870 (>C=CH_2) [6] and 2920, 2850, 1462 and 720 cm^{-1} (long chain). A molecular formula of $C_{47}H_{94}O_2$ was suggested from the $[M]^+$ at *m/z* 690 in its mass spectrum. Significant α-fission ions generated at *m/z* 649, 619, 71 and 41 located one hydroxyl group at C-3 and also showed the presence of a terminal isopropenyl function having unsaturation at C-1. Similarly the second hydroxyl group was placed at C-21 as the other α-fission ions were seen at *m/z* 381, 351, 339 and 309. These assignments were further supported by the ^1H NMR spectrum of **6** wherein the methyl group, comprising the isopropenyl function, resonated as a singlet at δ1.56 and the terminal double bond was seen as a multiplet at δ5.37 The downfield shift of the olefinic protons was possibly due to the adjacent C-3 hydroxyl group [7]. The C-3–CH(OH)–signal was, in turn, also shifted downfield to δ4.12 when compared to the C-21–CH(OH)– signal (δ3.65). The other end of the chain was terminated by a methyl group (δ0.90, *t*). These data were in complete agreement with the structure of **6** being 2-methylhexatetracont-1-en-3,21-diol.

Compound **4**, mp 75°, ν_{max} 2960, 2850, 1460, 725, 715 (long chain), 1370 (Me), 1732 and 1260 cm^{-1} (ester group) showed in its ^1H NMR spectrum two triplets at δ3.85 (–CH_2OCO–) and δ2.26 (–$COCH_2$). Alkaline hydrolysis of **4** afforded tritriacontanol (mp, mmp, IR, MS) and octacosanoic acid (mp, mmp, IR, MS) [8]. Compound **4**, therefore, was characterized as tritriacontanyl octa-

*Part 2 in the series "Studies on Indian Ginseng". For part 1 see ref. [1].



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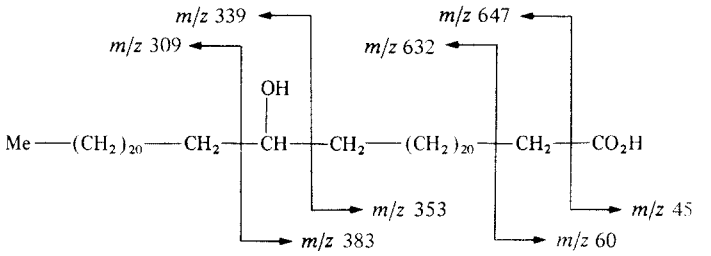

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cosanoate. The molecular ion was not seen in the mass spectrum of **4**, but the fragment ions for acid and alcohol were present.

Ginsenosides Rb_1 and Ro have a broad spectrum of biological activities [9–11]. It is interesting to note that **6** is a diol with a terminal isopropenyl moiety and is different from other polyunsaturated alcohols isolated from *Panax* spp. [7, 12–14]. Hydroxy acids occur in brain lipids, wool wax, milk lipids, cutins, micro-organisms and plants. Some examples of plant origin include ricinoleic acid, lesquerolic acid, dimorphecolic acid, aleuritic acid [15] and 15-hydroxyheneicosanoic acid [16].

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded in KBr and 80 MHz ^{1}H NMR spectra of **4** and **6** were measured in $CDCl_3$ and that of **7** in C_5D_5N, with TMS as internal standard. TLC was carried out on silica gel-60 precoated plates, F-254 (E. Merck). Visualization was effected by exposure to I_2 vapours or with 50% H_2SO_4 spray. Plant material was collected from the Singalila range of Darjeeling, West Bengal and identified in our Botany Department where a voucher specimen has been deposited.

Extraction and isolation of compounds. Dried and powdered rhizomes (268 g) of *P. pseudo-ginseng* subsp. *himalaicus* var. *angustifolius* were extracted with EtOH (50%, 9 × 600 ml). The solvent was removed *in vacuo* and the residue dissolved in EtOH (50%, 300 ml). It was then successively extracted with *n*-hexane (6 × 200 ml, 3 g) and *n*-BuOH (7 × 200 ml, 68 g). The residue from the *n*-hexane extract was chromatographed over silica gel (65 g, 60–120 mesh, B.D.H.), eluting with *n*-hexane, *n*-hexane–C_6H_6 (3:1, 1:1, 1:3), C_6H_6, C_6H_6–$CHCl_3$ (3:1, 1:1, 1:3), $CHCl_3$, $CHCl_3$–MeOH (19:1) and MeOH. Fractions (25 ml each) were collected and monitored by TLC. Similarly, part of the *n*-BuOH extract (40 g) was chromatographed over silica gel (1.2 kg), eluting with $CHCl_3$–MeOH (99:1, 95:5, 90:10, 80:20, 1:1). Fractions (250 ml each) were collected and each monitored by TLC. The homogeneity of all compounds was checked in at least two different solvent systems.

Ginsenoside-Rb$_1$ (**1**). Removal of solvent from $CHCl_3$–MeOH (1:1) fractions afforded a residue (8 g) which showed the presence of six saponins (three major, three minor). Part of this mixture (1 g) was subjected to repeated preparative TLC in $CHCl_3$–MeOH–H_2O (65:35:10, lower phase). Three polar saponins (major, R_f between 0.05 and 0.25) were separated and purified by preparative TLC in the same system. The upper zone of the three yielded a residue, 5 mg, mp 198° (MeOH), $[\alpha]_D$ + 14° (MeOH), identified as **1** by comparison with an authentic sample (mp, co-TLC).

Chikusetsusaponin-IVa (**2**). The middle zone from the above preparative TLC, afforded a residue, 15 mg, mp 220° (*n*-BuOH–H_2O–EtOAc), $[\alpha]_D$ + 21° (MeOH), identified by comparison with authentic material (mp, co-TLC). Compound **2** (12 mg) was hydrolysed with methanolic HCl (5%, 10 ml, 5 hr) to yield oleanolic acid (mp, mmp, co-TLC, $[\alpha]_D$, MS), glucuronic acid and glucose (co-PC, *n*-BuOH–AcOH–H_2O, 4:1:5).

Ginsenoside-Ro (**3**). The lower zone from the above preparative TLC provided a residue, 20 mg, mp 240–241° (MeOH), $[\alpha]_D$ + 16° (MeOH), identified by comparison with an authentic sample (mp, co-TLC) and from its hydrolysis products, oleanolic acid (mmp, $[\alpha]_D$, MS, NMR), glucuronic acid and glucose (co-PC).

Tritriacontanyl octacosanoate (**4**). Removal of solvent from fraction 15 of the *n*-hexane eluate yielded a residue, 10 mg, mp 75° ($CHCl_3$–MeOH). IR ν_{max} cm^{-1}: 2960, 2920, 2850, 1732, 1460, 1370, 1260, 1170, 725, 715. ^{1}H NMR: δ0.85 (6H, *t*, *J* = 6 Hz, 2

× terminal Me), 1.22 (112H, *br s*, $(CH_2)_{56}$), 3.85 (2H, *t*, $J = 6$ Hz, $-CH_2OCO-$), 2.26 (2H, *t*, $J = 6$ Hz, $-OC-CH_2-$). MS *m/z* (rel. int.): $[M]^+$ absent, 480 (0.3), 462 $[480 - H_2O]^+$ (0.6), 434 $[462 - 28]^+$ (0.6), 425 (0.2), 424 (2), 410 (0.4), 406 (0.4), 392 (2), 382 (1), 378 (0.3), 368 (2), 350 (0.5), 322 (0.5), 294 (0.5), 266 (1), 238 (1), 210 (1), 182 (2), 154 (3), 127 (8), 113 (10), 99 (16), 85 (40), 71 (65), 60 (10), 57 (100), 55 (41), 43 (78). Compound **4** (7 mg) was hydrolysed with ethanolic KOH (5%, 5 ml, 5 hr). When the reaction was complete, the mixture was diluted with H_2O (50 ml) and after work-up it afforded tritriacontanol (mp, IR, MS) and octacosanoic acid (mp, IR, MS).

Tritriacontanol (**5**). *n*-Hexane–C_6H_6 (1:1) fractions (73–74) yielded a residue, 5 mg, identified as tritriacontanol by co-TLC, mmp, MS, IR.

2-*Methylhexatetracont*-1-*en*-3,21-*diol* (**6**). The fast moving compound obtained by preparative TLC ($CHCl_3$–MeOH, 19:1) of the $CHCl_3$–MeOH (19:1) eluates (219–231) provided an amorphous residue, 8 mg. IR ν_{max} cm^{-1}: 3330, 3220, 2920, 2850, 1620, 1462, 1400, 1360, 1065, 1020, 870, 720. ^1H NMR: δ0.90 (3H, *t*, $J = 7$ Hz, H_3-46), 1.25 (76H, *br s*, $(CH_2)_{38}$), 1.56 (3H, *s*, H_3-2), 5.37 (2H, *m*, H_2-1), 4.12 (1H, *m*, H-3), 3.65 (1H, *m*, H-21), 2.05 (6H, *m*, H_2-4, H_2-20, H_2-22). MS *m/z* (rel. int.): 690 $[M]^+$ ($C_{47}H_{94}O_2$) (2), 675 (5), 649 (4), 619 (4), 381 (5), 351 (6), 339 (16), 309 (5), 239 (3), 225 (5), 127 (3), 113 (7), 99 (11), 85 (20), 71 (32), 57 (82), 55 (60), 43 (100), 41 (42).

24-*Hydroxyhexatetracontanoic acid* (**7**). The slow moving component from the preparative TLC of **6** furnished a residue, mp 122° ($CHCl_3$–Me_2CO), 15 mg. IR ν_{max} cm^{-1}: 3340, 3300–2500, 2920, 2850, 1715, 1465, 1375, 1275, 1065, 1020, 720. ^1H NMR: δ0.90 (3H, *t*, $J = 6$ Hz, H_3-46), 1.24 (80H, *br s*, $(CH_2)_{40}$), 2.25 (2H, *t*, $J = 6$ Hz, H_2-2), 2.05 (4H, *m*, H_2-23, H_2-25), 3.65 (1H, *m*, H-24). MS *m/z* (rel. int.): 692 $[M]^+$ ($C_{46}H_{92}O_3$) (0.1), 647 (3), 632 (3), 383 (3), 353 (2), 339 (10), 309 (3), 279 (5), 225 (6), 113 (8), 99 (10), 85 (20), 83 (45), 71 (35), 69 (46), 60 (65), 57 (76), 55 (70), 45 (6), 44 (20), 43 (100).

Acknowledgements—We wish to thank Dr. Akhtar Husain, Director, for his constant encouragement during the course of these studies and to Professor O. Tanaka, Hiroshima University, Japan for authentic ginsenosides. This work was supported by the International Foundation for Science (IFS), Stockholm, Sweden, Project No. 535.

REFERENCES

1. Shukla, Y. N. and Thakur, R. S. (1985) *Phytochemistry* **24**, 1091.
2. Sanada, S., Kondo, N., Shoji, J., Tanaka, O. and Shibata, S. (1974) *Chem. Pharm. Bull.* **22**, 421.
3. Kondo, N., Shoji, J. and Tanaka, O. (1973) *Chem. Pharm. Bull.* **21**, 2702.
4. Lin, T. D., Kondo, N. and Shoji, J. (1976) *Chem. Pharm. Bull.* **24**, 253.
5. Silverstein, R. M., Basler, G. C. and Morril, T. C. (1974) *Spectrometric Identification of Organic Compounds*, p. 27. John Wiley, New York.
6. Nakanishi, K. and Solomon, P. H. (1977) *Infrared Absorption Spectroscopy*, p. 17. Holden-Day, San Francisco.
7. Dabrowski, Z., Wrobel, J. T. and Wojtasiewicz, K. (1980) *Phytochemistry* **19**, 2464.
8. Shukla, Y. N. and Thakur, R. S. (1983) *Phytochemistry* **22**, 973.
9. Saito, H., Lee, Y., Takagi, K., Shibata, S., Shoji, J. and Kondo, N. (1977) *Chem. Pharm. Bull.* **25**, 1017.
10. Tanaka, O. and Kasai, R. (1984) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisewach, H., Kirby, G. W. and Tamm, Ch., eds) Vol. 46, p. 64. Springer, Vienna.
11. Lee, Y., Saito, H., Takagi, K., Shibata, S., Shoji, J. and Kondo, N. (1977) *Chem. Pharm. Bull.* **25**, 1391.
12. Takahashi, M. and Yoshikura, M. (1966) *Yakugaku Zasshi* **86**, 1051, 1053.
13. Poplawaski, J., Wrobel, J. T. and Glinka, T. (1980) *Phytochemistry* **19**, 1539.
14. Shim, S., Koh, H. and Han, B. (1983) *Phytochemistry* **22**, 1817.
15. Gunstone, F. D. (1979) in *Comprehensive Organic Chemistry* (Haslam, E., ed.) Vol. 5, p. 594. Pergamon Press, Oxford.
16. Goswami, A., Shukla, Y. N. and Thakur, R. S. (1981) *Phytochemistry* **20**, 1315.