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(Received September 5, 1979)

The branching positions in the glucuronide moieties of desacyl-jegosaponin (1), desacyl-boninsaponin A (2), and sakuraso-saponin (3) have been reinvestigated. It has been found that the branching positions in the glucuronide moieties of these glucuronide saponins should be revised to C-2 and C-3 from the previously assigned C-2 and C-4.

Keywords—glucuronide saponin; desacyl-jegosaponin; desacyl-boninsaponin A; sakuraso-saponin; methylated monosaccharide; GLC; TLC

Several years ago, we reported the structures of three glucuronide saponins: desacyl-jegosaponin from the pericarps of Styrex japonica Sieb. et Zucc. (Syracaceae),3) desacyl-boninsaponin A from the bark of Schima mertensiana Koz. (Theaceae),4) and sakuraso-saponin from the root of Primula sieboldi E. Morren (Primulaceae).5) In these studies, branching points at C-2 and C-4 of the glucuronide moieties in the oligosaccharide portions were assumed, based on the identification of methyl 3-O-methylglucopyranoside (i), which was obtained by methanolation of the lithium aluminum hydride (LiAlH₄) reduction products of the permethylated saponins. We have since utilized these glucuronide saponins as substrates for developing new selective cleavage methods for the glucuronide linkage in oligoglycosides.6)

However, in the course of our recent study on the saponins from the seeds of Aesculus turbinata Blume (Hippocastanaceae),7) we found that the previous identification methods (gas-liquid and thin-layer chromatography (GLC, TLC)3–5) for methyl 3-O-methylglucopyranoside (i) was not precise enough to distinguish i from methyl 4-O-methylglucopyranoside (ii).8) Therefore, the branching positions in the glucuronide moieties of the above-mentioned glucuronide saponins required reinvestigation.

The fully methylated derivatives (1a, 2a, 3a) of the three saponins were reduced with LiAlH₄ (giving 1b, 2b, 3b) and then methylated by Kuhn’s procedure9) to afford the corre-

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6) I. Kitagawa and M. Yoshikawa, Heterocycles, 8, 733 (1977), and references cited therein.
7) I. Kitagawa, K. Kobayashi, and M. Yoshikawa, to be published.
8) It has been found recently that i and ii can be distinguished from each other by TLC using silica gel 60F₂₅₄ (Merck) using double development with benzene–acetone (1: 1) (Rf: i=0.30; ii=0.24 (major), 0.27).
sponding glucosidic derivatives (1c, 2c, 3c). When these methylated products (1c, 2c, 3c) were subjected to methanalysis with hydrogen chloride in dry methanol, methyl 2,3,4-tri-O-methylrhamnopyranoside (iii), methyl 2,3,4,6-tetra-O-methylglucopyranoside (iv), methyl 3,4,6-tri-O-methylgalactopyranoside (v), and methyl 4,6-di-O-methylglucopyranoside (vi) were obtained as methylated monosaccharides from 1c and 2c, while iii, iv, and vi (as from 1c and 2c) were obtained from 3c.

Chart 1

1: R₁ = COOH, R₂ = R₃ = H, R₄ = OH (desacyl-jegosaponin)
   1a: R₁ = COOCH₃, R₂ = CH₃, R₃ = H, R₄ = OCH₃
   1b: R₁ = CH₂OH, R₂ = CH₃, R₃ = H, R₄ = OCH₃
   1c: R₁ = CH₂OCH₃, R₂ = CH₃, R₃ = H, R₄ = OCH₃

2: R₁ = COOH, R₂ = H, R₃ = OH, R₄ = H (desacyl-boninsaponin A)
   2a: R₁ = COOCH₃, R₂ = CH₃, R₃ = OCH₃, R₄ = H
   2b: R₁ = CH₂OH, R₂ = CH₃, R₃ = OCH₃, R₄ = H
   2c: R₁ = CH₂OCH₃, R₂ = CH₃, R₃ = OCH₃, R₄ = H

3: R₁ = COOH, R₂ = H (sakuraso-saponin)
   3a: R₁ = COOCH₃, R₂ = CH₃
   3b: R₁ = CH₂OH, R₂ = CH₃
   3c: R₁ = CH₂OCH₃, R₂ = CH₃

Chart 2

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1) C₆H₆CHO/ZnCl₂
2) C₆H₆NCO/py.

5: R = H
6: R = CNHC₆H₅

7: R₁ = CNHC₆H₅
   R₂ = H
8: R₁ = CN(CH₃)₃C₆H₅
   R₂ = H
9: R₁ = CH₃, R₂ = CH₃

Chart 3
Since methyl 4,6-di-O-methylglucopyranoside (vi) rather than methyl 3,6-di-O-methylglucopyranoside (viii) has been identified as the methylated monosaccharide deriving from the glucuronide moiety in the parent saponins, the branching positions in the glucuronide moieties of these saponins must be at C-2 and C-3 rather than the previously assumed C-2 and C-4. Taking into consideration our previous work\(^9\) to\(^{10}\) together with the present results, it is evident that the branching positions in the glucuronide moieties of desacetyl-jegosaprin, desacetyl-boninsaprin A, and sakuraso-saponin should be revised to those shown in the structures 1, 2, and 3, respectively (previous branching positions were erroneously given as C-2 and C-4).

An authentic sample of methyl 4,6-di-O-methylglucopyranoside (vi) was synthesized as shown in Chart 3. Methyl 4,6-O-benzylidene-\(\alpha\)-D-glucopyranoside (5)\(^{10}\) prepared from methyl \(\alpha\)-D-glucopyranoside (4) was converted to the phenylurethane (6) by treatment with phenylisocyanate in pyridine. Removal of the protecting benzylidene group in 6 followed by methylation\(^9\) gave 8. The infrared (IR) spectrum of 8 shows no absorption band due to NH, while the proton magnetic resonance (\(^1\)H-NMR) spectrum shows signals due to three O-methyls and two N-methyls. These \(^1\)H-NMR data, together with the mass spectral fragmentation pattern of 8,\(^{11}\) support the proposed structure. Finally, LiAlH\(_4\) reduction of 8 yielded methyl 4,6-di-O-methyl-\(\alpha\)-D-glucopyranoside (9),\(^{12}\) which was then subjected to methanolysis and found to be identical by GLC and TLC with vi liberated from 1c, 2c, and 3c as mentioned above.

In our previous reports on selective cleavage methods for the glucuronic linkage in oligoglycosides,\(^9\) the reaction pathways were discussed on the basis of previously proposed structures of desacetyl-jegosaprin and sakuraso-saponin. We are currently reinvigorating these results on the basis of the revised branching structures, and the results will be reported in due course.\(^{13}\)

### Experimental\(^{14}\)

**Hexadeca-O-methyl Derivative (1c) from Desacetyl-jegosaprin (1) — As reported previously,\(^9\) desacetyl-jegosaprin (1, 1 g) was methylethylated by Hakomori's method\(^{10}\) to give 1a (650 mg), which was then reduced with LiAlH\(_4\) in dry ether to furnish 1b (600 mg). A solution of 1b (500 mg) in dimethylformamide (DMF) (8 ml) was treated with CH\(_3\)I (15 ml) and Ag\(_2\)O (4.2 g) and stirred at 32° for 24 hr. After removing the inorganic material by filtration, the solution was diluted with ether and washed with water. The organic layer was separated, dried over MgSO\(_4\), and concentrated under reduced pressure. Preparative TLC (benzene-acetone=3:1) furnished 1c (340 mg) and 1b (130 mg, recovered). 1c, white powder, \(|\delta|_{\text{p}}^{25} -25.7° (\text{c}=1.0, \text{CCl}_4).\) Anal. Calcd for C\(_{36}\)H\(_{40}\)O\(_4\): C, 62.38; H, 9.13. Found: C, 62.20; H, 9.12. IR \(\nu_{\text{max}}\) cm\(^{-1}\): 2925, 1103, 1084. \(^1\)H-NMR (CDCl\(_3\), 6): 0.88—0.92 (15H), 1.04, 1.34 (3H each, both s) (tart. CH\(_2\)t), 1.25 (3H, d, J=5.5 Hz, sec. CH\(_3\)), 3.28, 3.32, 3.37, 3.40 (3H each, all s), 3.94—5.35 (36H) (OCH\(_3\times16\), 4.27, 4.59, 4.98 (1H each, all d, J=7 Hz), 5.17 (1H, s) (anomeric H×4), 5.26 (1H, br, s, W\(_{2s}=8\) Hz (olefinic H).**

**Hexadeca-O-methyl Derivative (2c) from Desacetyl-boninsaprin A (2) — Desacetyl-boninsaprin A (2, 500 mg) was methylethylated\(^{10}\) to give 2a (280 mg), which was then reduced with LiAlH\(_4\) in dry ether to afford 2b (240 mg) as reported previously.\(^9\) A solution of 2b (200 mg) in DMF (5 ml) was treated with CH\(_3\)I (10 ml) and Ag\(_2\)O (2 g) at 32° with stirring for 24 hr. After work-up as described for 1c, the product was purified by preparative TLC (benzene-acetone=3:1) to furnish 2c (140 mg) and 2b (20 mg recovered). 2c, white powder, \(|\delta|_{\text{p}}^{25} -22.6° (\text{c}=1.0, \text{CCl}_4).\) Anal. Calcd for C\(_{36}\)H\(_{40}\)O\(_4\): C, 62.38; H, 9.13. Found: C, 62.56; H, 9.02. IR \(\nu_{\text{max}}\) cm\(^{-1}\): 2930, 1102. \(^1\)H-NMR (CDCl\(_3\), 6): 0.85—0.92 (3H, s), 0.90—1.00 (15H), 1.25 (3H, s) (tart. **

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\(^{14}\) The instruments used to obtain the physical data, and the experimental conditions for chromatography were the same as in our previous paper\(^{13}\) unless otherwise specified.


\(^{16}\) S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
CH₃ x 7), 1.28 (3H, d, J = 6 Hz) (sec. CH₂), 3.25 (3H, s), 3.32, 3.34, 3.36, 3.39, 3.40 (6H each, all s), 3.47—3.52 (15H) (OCH₂ x 16), 4.13 (1H, d, J = 7 Hz), 4.50 (1H, d, J = 8 Hz), 4.87 (1H, d, J = 7 Hz), 4.91 (1H, s) (anomeric H x 4), 5.28 (1H, br, s, W₁₂ = 9 Hz) (olefinic H).

**Pentadeca-O-methyl derivative (3c) from Sakuraso-saponin (3)** — Sakuraso-saponin (3, 1 g) was methylated and the product (3a, 700 mg) was reduced with LiAlH₄ in dry ether to give 3b (640 mg) as reported previously. 3b (500 mg) in DMF (8 ml) was treated with CH₃I (15 ml) and Ag₂O (4.2 g) as described for 1c, and the product was purified by preparative TLC (benzene-acetone = 3:1) to furnish 3c (320 mg) and 3b (160 mg, recovered).

3c, white powder, [x]₂⁰ = -55.7° (c = 1.2, CCl₄). Anal. Calcd for C₂₃H₁₆O₅S: C, 62.22; H, 9.05. Found: C, 61.86; H, 9.05. IR ν₂₉⁰ cm⁻¹: 2930, 1100. ¹H-NMR (CDCl₃, δ): 0.87 (9H, s), 0.98, 1.01, 1.09, 1.15 (3H each, all s) (tetra-CH₂), 1.24, 1.27 (3H each, both d, J = 5.5 Hz) (sec. CH₂ x 2), 2.33, 3.30 (3H each, both s), 3.38 (6H, s), 3.49—3.59 (33H) (OCH₃ x 15), 4.24, 4.54, 4.96 (1H each, all d, J = 7 Hz), 5.02, 5.07 (1H each, both s) (anomeric H x 5).

**Methanalysis of 1c, 2c, and 3c** — A solution of 1c, 2c, or 3c (10 mg) in 9% HCl-dry MeOH (2 ml) was heated under reflux for 2 hr. The reaction mixture was neutralized with Ag₂CO₃ powder and filtered to remove inorganic material. The filtrate was concentrated under reduced pressure and the residue was analyzed by GLC and TLC.

From 1c and 2c, methyl 2,3,4,6-tetra-O-methylglucopyranoside (iv), methyl 2,3,4,6-tetra-O-methylglucopyranoside (iv), and methyl 4,6-dio-O-methylgluco-

**Phenylethane (6) from n-Glucose** — A solution of n-glucose (16.2 g) in 3% HCl-dry MeOH (65 ml) was heated under reflux for 3 hr. The reaction mixture was left to stand at 4° and the resulting colorless needles (4, 7.4 g, 42% yield) were collected by filtration. Recrystallization from methanol gave a pure sample of 4, mp 164° (colorless needles), [x]₂⁰ = +139.6° (c = 2.8, H₂O) [lit.: 18] mp 165° (MeOH), [x]₂⁰ = +157° (H₂O).

A solution of 4 (15 g) in benzaldehyde (38 ml) was treated with ZnCl₂ (11.3 g) and stirred at room temperature (12°) for 6 hr. Cold water (330 ml) was added and the mixture was left to stand at 5° for 12 hr, treated with petr. ether (25 ml), and stirred for 30 min. The resulting white precipitates were collected by filtration to give 5 (20.1 g, 92%). Recrystallization from CHCl₃-ether furnished 5 as colorless needles of mp 163—164°, [x]₂⁰ = +119.6° (c = 1.1, CHCl₃). IR ν₂₉⁰ cm⁻¹: 3476, 3462, 3005, 2941, 1467. ¹H-NMR (CDCl₃, δ): 3.39 (3H, s, OCH₃), 4.87 (1H, d, J = 4.5 Hz, anomeric H), 5.46 (1H, s, benzal H), 7.21—7.51 (5H, m, phenyl H x 5) [lit.: 10] mp 163—164° (CHCl₃), [x]₂⁰ = +110° (CHCl₃).

A solution of 5 (14 g) in pyridine (100 ml) was treated with phenylisocyanate (26 ml) and the total mixture was heated under reflux for 2 hr. After cooling, the reaction mixture was diluted with MeOH (50 ml) and heated again under reflux for 10 min. The reaction mixture was then poured into ice-water and extracted with CHCl₃. The CHCl₃ extract was then taken and dried over MgSO₄. The product (60 g) obtained by removal of the excess solvent was purified by column chromatography (silica gel, Merck, 70—230 mesh, 2 kg, benzene-acetone = 80:1—40:1) to furnish 6 (22 g, 85%). 6, mp 214—217° (colorless needles from MeOH), [x]₂⁰ = +32.5° (c = 0.9, CHCl₃). Anal. Calcd for C₂₁H₁₆O₇N₂: C, 64.61; H, 5.42; N, 5.38. Found: C, 64.70; H, 5.32; N, 5.40. IR ν₂₉⁰ cm⁻¹: 3488, 1754, 1606, 1538, 1450, 1315. ¹H-NMR (δ: acetone, δ): 3.46 (3H, s, OCH₃), 4.87 (1H, d, d, J = 4.5 Hz, 10 Hz-2H), 5.06 (1H, d, d, J = 4.5 Hz, 1H), 5.49 (1H, d, J = 10, 10 Hz, 3H), 5.64 (1H, s, benzal H), 6.92—7.58 (15H, m, phenyl H x 15), 8.68, 8.98 (1H each, both s, W₁₂ = 4.5, 5.5 Hz, exchangeable with D₂O, NH x 2) [lit.: 10] mp 216—217° (MeOH), [x]₂⁰ = +40° (CHCl₃).

17) The Kf values for methylated monosaccharides which ran close to the top and bottom are omitted.
Methyl 4,6-Di-O-methyl-α-D-glucopyranoside (9)—A solution of 6 (2.2 g) in acetone (30 ml) was treated with 0.8% aq. HCl (8 ml) and heated under reflux for 2 hr. After neutralization with Amberlite IRA-400 (OH⁻ form, 10 ml), the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to yield a residue, which was crystallized from CHCl₃-n-hexane to furnish 7 (1.72 g, 98%) as colorless needles of mp 159-161°C, [α]D +55.8° (c=0.8, pyridine). *Anal.* Calcd for C₁₃H₂₂O₄N₄: C, 58.33; H, 5.59; N, 6.48. Found: C, 58.33; H, 5.47; N, 6.53. IR ν max cm⁻¹: 3432, 1740, 1603, 1531, 1447. ¹H-NMR (δ, acetone, δ): 3.39 (3H, s, OCH₃), 4.74 (1H, d, d, J=3.5, 11 Hz, 2-H), 4.96 (1H, d, J=3.5 Hz, 1-H), 5.25 (1H, m, 3-H), 6.84-7.53 (10H, m, phenyl H x 10), 8.67, 8.92 (1H each, both br.s, W H=3.5 Hz, NH x 2) [lit.¹]: mp 151-153°C (ligroin-AcOEt), [α]D +55° (pyridine).

A solution of 7 (600 mg) was treated with CH₃I (14.4 ml) and Ag₂O (5.4 g) and stirred at 31°C for 24 hr in the dark. After removing inorganic material by filtration, the filtrate was concentrated under reduced pressure. The product (660 mg) was purified by column chromatography (silica gel, 50 g, benzene-AcOEt =30:1-10:1) to furnish 8 (636 mg, 92%). 8, colorless oil, [α]D +128.2° (c=0.8, CHCL₃). High resolution mass spectrum; Calcd for C₁₃H₂₂O₄N₄: 488.216, C₁₃H₂₄O₄N₄ (δ): 326.125, C₁₃H₂₄O₄δ (δ): 102.068. Found: 488.214, 326.123, 102.068. IR ν max cm⁻¹: 1730, 1596, 1497, 1155, 1062. ¹H-NMR (CDCl₃, δ): 3.28, 3.32, 3.37 (3H each, s), 3.35 (6H, s) (OCH₃ x 3, NCH₂ x 2), 4.65 (1H, d, J=4, 9 Hz, 2-H), 4.97 (1H, d, J=4, 1-H), 5.39 (1H, d, J=9, 9 Hz, 3-H), 7.05-7.34 (10H, m, phenyl H x 10).

A suspension of LiAlH₄ (60 mg) in dry ether (5 ml) was added dropwise to a solution of 8 (270 mg) in dry ether (10 ml), and the whole was heated under reflux for 1 hr. After cooling, the reaction was terminated by adding aq. ether and the mixture was neutralized with Dowex 50w x 8 (H⁺ form, 5 ml). After removing the resin by filtration, the filtrate was concentrated under reduced pressure. The residue was then purified by preparative TLC (benzene-acetone=3:5) to furnish methyl 4,6-di-O-methyl-α-D-glucopyranoside (9, 106 mg, 86%). 9, colorless oil, [α]D +159.0° (c=1.1, CHCl₃). IR ν max cm⁻¹: 3404, 2918, 1069. ¹H-NMR (CDCl₃, δ): 3.43 (6H, s), 3.56 (3H, s) (OCH₃ x 3), 4.74 (1H, d, J=2 Hz, 1-H) [lit.¹]: [α]D +157° (CHCl₃). A solution of 9 in 9% HCl-dry MeOH (1.5 ml) was heated under reflux for 2 hr, then neutralized with Ag₂CO₃. The product obtained by concentration of the filtrate was used for identification of 9 by GLC and TLC as described above.