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THE CONSTITUTION OF SAPONINS ISOLATED FROM BUPLEURUM FALCATUM L.

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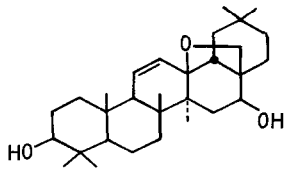
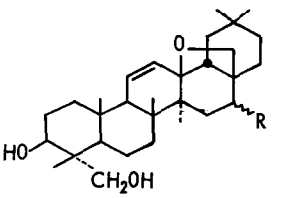
Recently, we have isolated saikogenins E (1, 2), F (3) and G (4) as the genuine sapogenins of the root of Bupleurum falcatum L. and demonstrated (4) that the parent saponins corresponding to these sapogenins are saikosaponins c, a and d (5), which on TLC using silica gel G and AcOEt-EtOH-H_2O (8 : 2 : 1 v/v) exhibit R_f 0.21, 0.34 and 0.38, respectively.



Saikogenin E



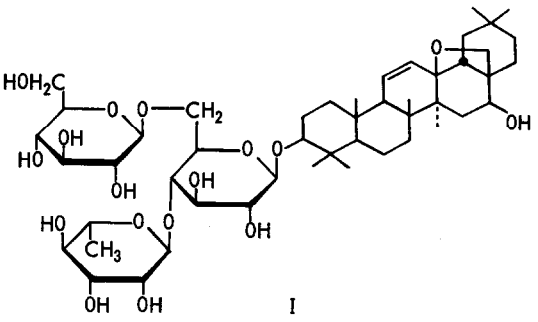
Saikogenin F R = β-OH
Saikogenin G R = α-OH

This paper deals with the structure elucidation of saikosaponins. The saponins were isolated as described previously (4) and separated by preparative TLC into the individual components, which were recrystallized from AcOEt-MeOH.

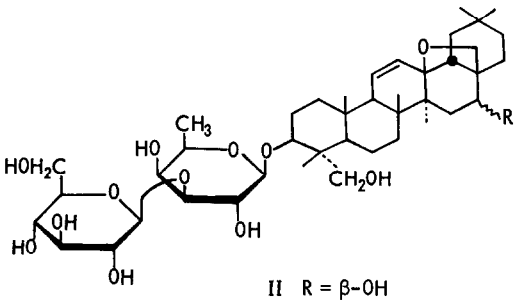
Saikosaponin c, m.p. 202-210°, $[\alpha]_D$ +4.3° (EtOH), on acid hydrolysis afforded D-glucose and L-rhamnose, of which the molar ratio was determined as 2 : 1 by GLC (6) of the TMS derivative of the hydrolysate. Full methylation of saikosaponin c was achieved by prolonged treatment with the Hakomori's procedure (7). After acid hydrolysis of the permethylate, the sugar fraction was identified with authentic specimens of 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose by GLC (8) and TLC. On the other hand, saikosaponin c on brief treatment with refluxing 0.1 N H_2SO_4 in 70% MeOH was transformed into a saponin having the conjugated diene as in saikogenin C (9),

without hydrolysis of the sugar moiety. Additional 4 hr treatment yielded a prosapogenin accompanied with release of the rhamnose, which was identified by GLC (6) and TLC. The prosapogenin was fully methylated by the Hakomori's method (7) and then hydrolyzed with HCl-MeOH. The sugar fraction was identified as 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose by GLC (8) and TLC. The aglycone part was identified by IR, GLC and TLC with authentic 16,28-di-O-methyl-saikogenin C, which was prepared by LAH reduction of 16β,28-dimethoxyolean-11,13(18)-dien-3-one, m.p. 138-140°, $[\alpha]_D$ -43°, derived from saikogenin C (9) through several steps. These experimental results have proved the connecting sequence of the sugar moiety in saikosaponin c and, with application of the Klyne's rule (10) for determination of the configuration of the glycoside linkages, the structure of saikosaponin c may be defined as saikogenin E 3-[β-D-glucopyranosyl(1→6)]-[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside (I).

Both of saikosaponins a, m.p. 225-232°, $[\alpha]_D$ +46° (EtOH), and d, m.p. 212-218°, $[\alpha]_D$ +37° (EtOH), on acid hydrolysis afforded D-glucose and D-fucose, of which the molar ratio was determined as 1 : 1 by the GLC method (6). As described earlier (4), the saponins a and d on degradation by oxidation with $NaIO_4$ followed by treatment with KOH gave prosaikogenins F, m.p. 246-250°, $[\alpha]_D$ +63° (EtOH), and G, respectively, which were shown to be fucosides of saikogenins F and G. In view of these findings, it is clear that the fucose moiety in the saponins a and d has no vicinal glycol owing to linkage with the glucose and therefore the D-glucose must link to the C_3-OH of the D-fucose. Treatment of saikosaponins a and d with refluxing 0.1 N H_2SO_4 in 70% MeOH resulted in the transformation of the aglycone moiety into the conjugated diene structure as in saikogenins A and D (9), without hydrolysis of the sugar part. The transformed saponins were fully methylated by the Hakomori's method (7) and then hydrolyzed with a mixture of 2 N H_2SO_4 and EtOH (1 : 2) under refluxing for 5 hr. Each of the water-soluble fractions obtained from both the saponins, after further hydrolysis with aq HCl and chromatography on Al_2O_3, yielded 2,3,4,6-tetra-O-methyl-D-glucose, m.p. 83-87°, $[\alpha]_D$ +92→+77° (H_2O), (lit. (11) m.p. 84-87°, $[\alpha]_D$ +78°) and 2,4-di-O-methyl-D-fucose, m.p. 131-134°, $[\alpha]_D$ +139→+93° (H_2O), (lit. (12) m.p. 131-134°, $[\alpha]_D$ +86°), as was expected. The water-insoluble precipitates from the saponins a and d afforded 16,23,28-tri-O-methyl-saikogenins A, m.p. 177-179°, $[\alpha]_D$ -62°, and D, m.p. 115°, $[\alpha]_D$ -98°, respectively, both of which were proved to have the free OH



I



II R = β-OH
III R = α-OH

at C_3 by the fact that the respective acetates m.p. 198-201°, $[\alpha]_D$ -49°, and m.p. 167-169°, $[\alpha]_D$ -85°, exhibit the same NMR signal at τ 5.05 (q., J=10, 6 c/s) attributable to 1 H on C_3 bearing the AcO group. From the above results, the connecting sequences of the sugar parts in saikosaponins a and d have been established and application of the Klyne's rule (10) may lead to the conclusion that the structure of saikosaponins a and d are represented as 3-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranosides of saikogenins F (II) and G (III), respectively.

Furthermore, permethylation of saikosaponin a followed by acid hydrolysis and acetylation yielded 3β,28-diacetoxy-16β,23-dimethoxy-olean-11,13(18)-diene, $C_{36}H_{56}O_6$, m.p. 174-179°, $[\alpha]_D$ -74°. This result rules out a possibility that the 13β,28-epoxy-Δ^{11}-ene function in saikogenins E, F and G might be produced from 11,28-dihydroxy-Δ^{12}-ene by action of formic acid during the $NaIO_4$ oxidation of the saponins (4) and verifies that saikogenins E, F and G are certainly genuine sapogenins of Bupleurum falcatum L.

In the previous experiments (4) on the isolation of saikogenins E, F and G by the periodate-alkali treatment of the saponins, saikogenin E was obtained in a reasonable yield while the yields of saikogenins

F and G were much inferior. With the establishment of the structure of saikosaponins, the above facts may be interpreted by the assumption that the aldehyde intermediate derived from the $NaIO_4$ oxidation of saikosaponin c has the structure favourable for the β-elimination of the glycoside linkage with alkali whereas those from saikosaponins a and d do not.

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5. Saikosaponin b was shown already (4) to be an artifact transformed from saikosaponin d during an ordinary extraction of the saponins. TLC developed with $CHCl_3$-MeOH-H_2O (30 : 10 : 1 v/v) is detectable saikosaponins a, b and c as the spots of R_f 0.40, 0.30 and 0.19, respectively, which were previously designated as saikosides Ia, Ib and II by Shibata et al. Cf. Tetrahedron Letters 3783 (1965); Chem. Pharm. Bull. 14, 1023 (1966). However, on this TLC system, saikosaponin d superimposes on saikosaponin a and the saponins, having the conjugated diene, transformed from saikosaponins a and c exhibit the same R_f values as those of the original a and c.
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