TRITERPENE GLYCOSIDES OF THE LEAVES OF Eleutherococcus senticosus

II. STRUCTURE OF ELEUTHEROSIDES I, K, L, AND M

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In the preceding communication we have described the isolation from the leaves of Eleutherococcus senticosus of four glycosides of oleanolic acid and have given preliminary information on their structure. The present paper gives a proof of the structures of the carbohydrate chains of eleutherosides I, K, L, and M by the methylation method.

The exhaustive methylation of the combined eleutherosides I and K by Kuhn's method [1] followed by chromatographic separation led to the isolation of the two fully methylated eleutherosides I and K. The same products were isolated in the methylation of the glycosides formed by the alkaline cleavage of eleutherosides L and M, respectively.

When methylated eleutheroside K was hydrolyzed, 2,3,4-tri-O-methyl-L-rhamnose and 3,4-di-O-methyl-L-arabinose were identified, while the hydrolysis of methylated eleutheroside I gave 2,3,4-tri-O-methyl-L-rhamnose and a di-O-methyl-L-arabinose differing from that mentioned above. Since there are no 1,3 bonds in the molecule of eleutheroside I, this may be 2,3-di-O-methyl-L-arabinose.

The above information and the results of a calculation of molecular rotation according to Klyne's rule [2] permit the following structures to be proposed for eleutherosides I and K:

$$\alpha - L - Rha\,(1-4)\,\alpha - L - \text{Ara}(1-3) - O - R - COOH$$
$$\alpha - L - Rha\,(1-2)\,\alpha - L - \text{Ara}(1-3) - O - R - COOH$$

where R–COOH is an oleanolic acid residue;
Rha is a rhamnopyranose residue; and
Ara is an arabopyranose residue.

Consequently, eleutheroside I is mubenin B, which has been described previously [3].

The hydrolysis of the fully methylated eleutheroside L gave 2,3,4-tri-O-methyl-L-rhamnose, 2,3-di-O-methyl-L-arabinose, and 2,3,4- and 2,3,6-tri-O-methyl-D-glucoses. The acid hydrolysate of methylated eleutheroside M contained 3,4-di-O-methyl-L-arabinose together with the methyl ethers of L-rhamnose and D-glucose mentioned above. The reduction of methylated eleutheroside L with lithium tetrahydroaluminate gave a bioside of erythrodiol and a trisaccharide. The hydrolysis of the bioside led to 2,3,4-tri-O-methyl-L-rhamnose and 2,3-di-O-methyl-L-arabinose. When the trisaccaride was hydrolyzed, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methylsorbitol were identified. A similar trisaccharide was formed in the reduction of methylated eleutheroside M. With the trisaccharide was isolated a bioside the hydrolysis of which gave 2,3,4-tri-O-methyl-L-rhamnose and 3,4-di-O-methyl-L-arabinose. Thus, eleutherosides L and M have the same carbohydrate chains attached to the carboxy group of the oleanolic acid, and the difference between them consists only in the nature of the carbohydrate chain at C-3 of the genin. On the basis of this and the calculation of molecular rotation by Klyne's rule, eleutherosides L and M have the structures

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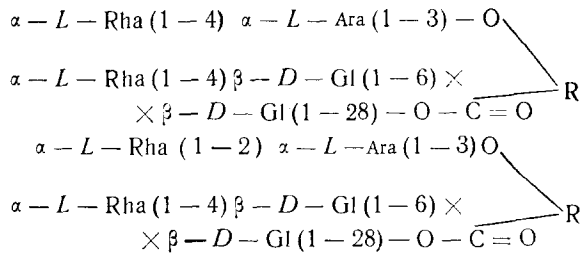

where R is an oleanolic acid residue.

Consequently, eleutheroside M is identical with the hederasaponin B described previously [4].

EXPERIMENTAL

Chromatography was performed on Whatman 3M paper in the following solvent systems: 1) butan-1-ol–ethanol–water (40 : 11 : 19); 2) methyl ethyl ketone saturated with 1% aqueous ammonia. The following systems were used for thin-layer chromatography (TLC): 3) chloroform–ethanol (22 : 0.8), 4) chloroform–ethyl acetate (18 : 3), 5) chloroform–acetone (7 : 1), 6) benzene–acetone (3 : 1).

The gas-liquid chromatography (GLC) of the methyl glycosides of the methylated monosaccharides was performed on a Tswett-2 chromatograph with a flame-ionization detector. A column containing 15% of butanediol succinate on Chromosorb W (40-60 mesh) washed with acid and treated with chlorodimethylsilane with a programmed temperature from 120 to 200°C at the rate of 5 deg/min was used.

Methylation of Eleutherosides I and K. The methylation of 0.5 g of combined eleutherosides I and K was performed in solution in 5 ml of dimethylformamide (DMFA) with 5 ml of methyl iodide in the presence of 1 g of silver oxide in an atmosphere of argon in the dark at 50-60°C for 40 h. During the process, new 5-ml portions of methyl iodide and 1-g portions of silver oxide were added three times. The reaction mixture was poured into chloroform, the precipitate was separated by filtration, and the filtrate was treated with a saturated solution of sodium thiosulfate, dried, and evaporated. This gave 0.55 g of a mixture of fully methylated eleutheroside I and K (the IR spectrum lacked the absorption band of a hydroxyl).

This mixture (0.5 g) was deposited on a column (2 × 30 ml) of silica gel and was eluted with chloroform containing increasing concentrations of methanol. The yield of methylated eleutheroside I was 70 mg $[\alpha]_D^{20}+3.2°$ (in chloroform) and that of K was 136 mg $[\alpha]_D^{20}+22.4°$ (in chloroform). Their IR spectra lacked the absorption band of hydroxyl. Similar compounds were formed by the methylation of eleutherosides I and K obtained by the alkaline hydrolysis of eleutherosides L and M, respectively.

Methylation of Eleutherosides L and M. The methylation of 0.50 g of eleutheroside L and of 0.52 g of eleutheroside M, each dissolved in 6 ml of DMFA, was performed as described above. The yield of fully methylated eleutheroside L was 0.55 g, $[\alpha]_D^{20}-11.5°$ (in chloroform) and that of fully methylated eleutheroside M 0.60 g, $[\alpha]_D^{20}-19°$ (in chloroform).

Methanolysis and Hydrolysis of the Methylated Eleutherosides. Methanolysis was performed with 5% $HClO_4$ in absolute methanol in sealed tubes at 100°C for 6-7 h. The reaction mixtures were treated with Dowex 1 × 4 resin (HCO_3^-) and evaporated. By GLC and TLC in systems 5 and 6 the methanolysates of eleutherosides I and K were each shown to contain the methyl glycosides of 2,3,4-tri-O-methylrhamnose and of a di-O-methylarabinose, the arabinose derivatives being different for the two eleutherosides. The methyl glycosides were hydrolyzed with 2 N sulfuric acid at 100°C for 6 h. The acid solutions were neutralized with barium carbonate, treated with Amberlite IR-120 (H^+), and evaporated. The methylated monosaccharides were chromatographed on paper in systems 1 and 2. The hydrolysates of eleutherosides I and K were found to contain 2,3,4-tri-O-methylrhamnose and, in addition, the hydrolysate of eleutheroside I contained 2,3-di-O-methylarabinose, which was not revealed by Bonner's reagent [5], unlike the 3,4-di-O-methylarabinose present in the hydrolysate from glycoside K. The methanolysates of the methylated eleutherosides L and M were investigated similarly. They contained the methyl glycosides of 2,3,4-tri-O-methylrhamnose and 2,3,4- and 2,3,6-tri-O-methylglucoses while the methanolysate of methylated L also contained methyl 2,3-di-O-methylarabinoside and the methanolysate of methylated eleutheroside M contained methyl 3,4-di-O-methylarabinoside. These results were confirmed by the PC of the corresponding hydrolysates.

The mixture of methyl glycosides (1 g) isolated from the methanolysis of 1.6 g of the combined methylated eleutherosides L and M was deposited on a column of silica gel (4 × 23 cm) and eluted with chloroform and then with increasing concentrations of methanol in chloroform. The fractions consisting of the methyl glycosides of the individual methylated monosaccharides were combined, evaporated, and hydrolyzed with 2 N sulfuric acid, as described above. The samples of methylated monosaccharides were additionally chromatographed on a column of cellulose (2 × 22 cm) in the water-saturated methyl ethyl ketone system. The methylated monosaccharides were identified by demethylation with boron tribromide [6] and identification of the free monosaccharides and by additional methylation [1] with the identification of the products obtained.

As a result we identified and compared with authentic samples 2,3,4-tri-O-methyl-L-rhamnose with $[\alpha]_D^{20}+28°$ (in water) and 2,3,4-tri-O-methyl-D-glucose with $[\alpha]_D^{20}+79°$ (in water) (on reduction with sodium tetrahydroborate and subsequent periodate oxidation, 2,3,4-tri-O-methylxylose was formed); 2,3,6-tri-O-methyl-D-glucose with $[\alpha]_D^{20}+70°$ (in water) (after reduction and periodate oxidation, 2,3-di-O-methylthreose, chromatographically identical with the 2,3-di-O-methylthreose obtained in the oxidation of 2,3,6-tri-O-methylsorbitol, was obtained); 3,4-di-O-methyl-L-arabinose with $[\alpha]_D^{20}+180°$ (in water) (revealed by Bonner's reagent); and 2,3-di-O-methyl-L-arabinose (not revealed with Bonner's reagent).

Reduction of the Methylated Eleutherosides L and M. To a solution of 0.45 g of methylated eleutheroside M in freshly distilled tetrahydrofuran (THF) was added 0.1 g of lithium tetrahydroaluminate. The mixture was stirred for 1 h and was then boiled for 4 h and left overnight. The excess of lithium tetrahydroaluminate was decomposed with water. The precipitate was separated off and was washed with THF and with ether. The aqueous layer was extracted with ether. The ethereal-tetrahydrofuran solutions were dried and evaporated to dryness. Yield 0.41 g. This residue was dissolved in ether, and the ethereal solution was extracted with water. The aqueous layer was evaporated to dryness. The yield of methylated reduced trisaccharide (I) was 67.3 mg, $[\alpha]_D^{20}-34.2°$ (in chloroform). The ethereal layer was evaporated to dryness. The resulting product was chromatographed on a column of silica gel (2.5 × 18 cm), being eluted with chloroform. The yield of the methylated bioside of erythrodiol (II) was 250 mg $[\alpha]_D^{20}+20.9°$ (in chloroform).

The methylated eleutheroside L (0.17 mg) was reduced and treated similarly. This gave the methylated reduced trisaccharide (III) with a yield of 20 mg, $[\alpha]_D^{20}-32.7°$ (in chloroform), and the methylated biosides (IV) with a yield of 86 mg, $[\alpha]_D^{20}+1.9°$ (in chloroform).

Hydrolysis of the Methylated Biosides (II) and (IV). The methanolysis and hydrolysis of the methylated biosides (II) and (IV) were performed by the usual method. By GLC, methyl 2,3,4-tri-O-methyl-L-rhamnoside and methyl 3,4-di-O-methyl-L-arabinoside were identified in the methylated bioside II, and methyl 2,3,4-tri-O-methyl-L-rhamnoside and methyl 2,3-di-O-methyl-L-arabinoside in the methylated bioside (IV). These results were confirmed by the PC of the corresponding hydrolysates in systems 1 and 2.

Hydrolysis of the Methylated Trisaccharides (I) and (III). The methylated trisaccharide (I) (67 mg) was hydrolyzed as described above. The mixture of methylated monosaccharides was chromatographed on a column of silica gel (1.5 × 9 cm), elution being performed with mixtures of chloroform and increasing amounts of acetone. 2,3,4-Tri-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4-tri-O-methylsorbitol were isolated and identified by comparison with authentic samples.

The hydrolysis of the methylated trisaccharide (III) gave similar results.

SUMMARY

The complete structures of eleutherosides I, K, L, and M have been established. Glycoside I is identical with mubenin B and glycoside M with hederasaponin B. It has been shown that eleutheroside K is the 3-[O-α-L-rhamnopyranosyl-(1→2)-α-L-arabopyranoside] of oleanolic acid. In eleutheroside L, the L-rhamnose and L-arabinose residues are connected by a 1→4 bond, and a trisaccharide consisting of O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranose is connected with the carboxy group.

LITERATURE CITED

1. R. Kuhn, H. Trischmann, and J. Low, Angew. Chem., 67, 32 (1955).
2. W. Klyne, Biochem. J., 47, xli (1950).

3. T. Takemoto and K. Kometani, Ann. Chem., 685, 237 (1965).
4. R. Tschesche, W. Schmidt, and G. Wulff, Z. Naturforsch., 20b, 708 (1965); Chem. Abstr., 63, 7050h (1965).
5. T. Bonner, Chem. Ind. (London), 1960, 345.
6. T. Bonner, E. Bourne, and S. McNally, J. Chem. Soc., 1960, 2929.