GLYCOSYLATION OF TRITERPENOIDS OF THE DAMMARANE SERIES. II. 12,25-DI-O- AND 3,12,25-TRI-O-GLUCOPYRANOSIDES OF 20(S),24(R)-EPOXYDAMMARANE-3α,12β,25-TRIOL AND ITS 3-EPIMER

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On the glycosylation of 20(S),24(R)-epoxydammarane-3α,12β,25-triol (I), its 3-O-acetyl derivative (II), and its 3-epimer with α-acetobromoglucose under the conditions of Helferich's modification and with glucose tert-butyl orthoacetate under the conditions of the orthoester method, di- and triglycosides, respectively, are obtained. The physicochemical constants and IR and PMR spectroscopic characteristics of the compounds obtained for the first time are given.

The synthesis of glycosides of triterpenoids of the dammarane series is of interest not only because of the structural closeness of these substances to the glycosides of ginseng [1-3], but also from the point of view of a study of the features of the glycosylation of such complex polyfunctional compounds. With this aim, and also in order to study biological activities, we have synthesized a diglucoside of 20(S),24(R)-epoxydammarane-3α,12β,25-triol (I), and the triglucosides of (I) and of its 3-epimer (IV).

Since in a preceding publication [4] we showed the possibility of introducing a carbohydrate component at a tertiary hydroxy group in triterpenoids of the dammarane series in the case of compound (I) as an example, we have carried out the glycosylation of the remaining hydroxy groups in this compound. Furthermore, as another starting material we have used 20(S),24(R)-epoxydammarane-3β,12β,25-triol (IV), since in natural glycosides with such a structure of the aglycone the hydroxy group at C_3 is equatorial [3].

Glycosylation was effected by two methods: by the orthoester method [5] and by Helferich's modification [6]. The results are given in Table 1. The main reaction products were fully acetylated glucosides (V-VII) (see Table 1). The structures of the compounds obtained were confirmed by their spectral characteristics and elementary analyses. The IR spectra of compounds (V-VII) contained no bands of the stretching vibrations of hydroxy groups.

The PMR spectra of compound (V) contained two, and each of those of compound (VI) and (VII) three, doublet signals of anomeric protons with $J_{1,2}$ = 7.5-8 Hz, showing the β configuration of all the glycosidic bonds in compounds (V), (VI) and (VII).

Compound (V) was deacetylated with 10% KOH in methanol, and compounds (VI) and (VII) with sodium methanolate in methanol, giving 90-95% yields of the free glucosides (VIII, IX, and X).

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrophotometer and PMR spectra on a Brüker HX-90E spectrometer in deuterochloroform with tetramethylsilane as internal standard.

Optical rotations were determined on a Perkin-Elmer-141 polarimeter at 20°C, and melting points on a Boëtius stage. Column chromatography was performed on KSK silica gel (80-120 mesh) in the petroleum ether—acetone system.

Then individuality of the substances was checked with the aid of TLC on silica gel in systems: 1) hexane—acetone (2:1) and 2) benzene—chloroform—methanol (2:1:1).

The substances were revealed with 10% sulfuric acid in methanol followed by heating at 100-200°C. Chlorobenzene and nitromethane were purified as described in the literature [5].

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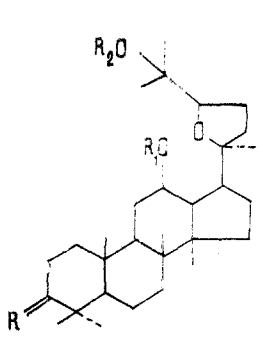

I. R =  , $R_1=R_2=H$

II. R =  , $R_1=R_2=H$

III. R=O, $R_1=R_2=H$

IV. R =  , $R_1=R_2=H$

V. R =  , $R_1=R_2=Glc(Ac)_4$

VI. R =  , $R_1=R_2=Glc(Ac)_4$

VII. R =  , $R_1=R_2=Glc(Ac)_4$

VIII. R =  , $R_1=R_2=Glc$

IX. R =  , $R_1=R_2=Glc$

X. R =  , $R_1=R_2=Glc$

The results of elementary analysis for all the newly obtained compounds coincided with the calculated figures.

General Procedure for Performing Condensations with D-Glucose tert-Butyl Orthoacetate

2,4,6-Collidinium perchlorate (4-8 mg) in 10 ml of chloroform was dried by azeotropic distillation, and then 1 mmole of a hydroxyl-containing compound was added to the solution and another few milliliters of solvent was distilled off. The necessary amount of D-glucose tert-butyl orthoacetate (see Table 1) was added in two or three portions at intervals of 5-10 min under the conditions of azeotropic distillation. After the end of the reaction (checked by TLC in system 1), the reaction mixture was evaporated. The dry residue was chromatographed on silica gel.

General Procedure for Performing Condensations with α-Acetobromoglucose

A mixture of 1 mmole of hydroxyl-containing compound, the necessary amount of α-acetobromoglucose (see Table 1), and mercury cyanide in 10 ml of absolute nitromethane was stirred at room temperature for 4-5 h. Then the reaction mixture was evaporated, the residue was dissolved in chloroform, and the solution was filtered from mercury salts. The filtrate was washed with water, dried with anhydrous sodium sulfate, and evaporated. The dry residue was chromotographed on silica gel.

20(S),24(R)-Epoxydammarane-3α,12β,25-triol (Betulafolienetriol Oxide) (I). This was isolated from the unsaponifiable fraction of an ethereal extract of *Betula mandshurica* with subsequent purification on silica gel. It was crystallized from acetone, mp 235-237°C. According to the literature [7]: mp 237-240°C.

3α-Acetoxy-20(S),24(R)-Epoxydammarane-12β,25-diol (II). This was obtained by the acetylation of (I) with acetic anhydride in pyridine at room temperature for 4-5 h followed by purification on silica gel; $[\alpha]_D^{20}$ −14.8° (c 0.3; chloroform). $C_{32}H_{54}O_3$.

IR spectra ($CHCl_3$, cm^{-1}): 1220, 1720 (COOR), 3420, 3620.

TABLE 1

Initial substances, mmole			Yield* of the main substance, %
Alcohol	Glycosylating agent	Catalyst	
II	D-Glucose tert-butyl orthoacetate, 3	2,4,6-Collidinium perchlorate	(V) 47
II	α-Acetobromoglucose, 3	$Hg(CN)_2$, 3	(V) 57.1
I	D-Glucose tert-butyl orthoacetate, 4	2,4,6-Collidinium perchlorate	(VI) 36.2
I	α-Acetobromoglucose, 4	$Hg(CN)_2$, 4	(VI) 40.1
IV	α-Acetobromoglucose, 4	$Hg(CN)_2$, 4	(VII) 53.6

*The yields are given on the chromatographically homogeneous substances.

PMR spectrum (δ, ppm): 0.83-1.27 (3 H × 8, s) — the protons of methyl groups; 2.08 (3 H, s) — the protons of an acetyl group; 3.53 (1 H, d—t) — proton at C_{12}; 3.85 (1 H, m) — proton at C_{24}; 4.64 (1 H, t, J = 3 Hz) — proton at C_3.

12β,25-Dihydroxy-20(S),24(R)-epoxydammaran-3-one (III). This was obtained by the oxidation of (I) with chromium trioxide in pyridine $[\alpha]_D^{20}$ +35.3° (c 1.0; chloroform), $C_{30}H_{51}O_4$.

IR spectrum ($CHCl_3$, cm^{-1}): 1696 (C=O), 3420, 3620.

PMR spectrum (δ, ppm): 0.92-1.28 (3 H × 8, s) — protons of methyl groups; 3.52 (1 H, d—t) — proton at C_{12}; 3.86 (1 H, m), proton at C_{24}.

20(S),24(R)-Epoxydammarane-3β,12β,25-triol (IV). This was obtained by the reduction of (III) with sodium tetrahydroborate in isopropanol. It was crystallized from acetone; mp 218-220°C, $[\alpha]_D^{20}$ +12.0° (c 1.0; chloroform).

IR spectrum ($CHCl_3$, cm^{-1}): 3300, 3397, 3590.

PMR spectrum (δ, ppm): 0.77-1.27 (3 H × 8, s) — protons of methyl groups; 3.20 (1 H, m) — protons at C_3; 3.53 (1 H, d—t) — protons at C_{12}; 3.85 (1 H, m) — proton at C_{24}.

3α-Acetoxy-12β,25-di(2',3',4',6'-tetra-O-acetyl-β-D-glycopyranosyloxy)20(S),24(R)-epoxydammarane (V). mp 157-160°C (ethanol), $[\alpha]_D^{20}$ — 23.5° (c 1.0; chloroform).

IR spectrum ($CHCl_3$, cm^{-1}): 1725, 1754, 1220, 1260 (COOR).

PMR spectrum (δ, ppm): 0.85-1.14 (3 H × 8, s) — protons of methyl groups; 1.99-2.09 (3 H × 9, s) — protons of acetyl groups; 2.69 (1 H, m) — proton at C_{24}; 4.63 (1 H, d, $J_{1,1}$ = 7.6 Hz), 5.07 (1 H, d, $J_{1,2}$ = 7.9 Hz) — anomeric protons.

3α,12β,25-Tri(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyloxy)-20(S),24(R)-epoxydammarane (VI). mp 169-171°C (ethanol); $[\alpha]_D^{20}$ — 17.0° (c 1.0; chloroform), $C_{72}H_{106}O_{31}$.

PMR spectrum (δ, ppm): 0.84-1.14 (3H × 8, s) — protons of methyl groups; 2.02-2.08 (3 H × 12, s) — protons of acetyl groups; 3.64 (1 H, m) — protons at C_{24}; 5.06 (1 H, d, $J_{1,2}$ = 8.2 Hz) — proton at the anomeric carbon atom of the glucose residue at C_{25}; 4.46 (1 H, d, $J_{1,2}$ = 7.5 Hz) and 4.38 (1 H, d, $J_{1,2}$ = 7.5 Hz) — protons at the anomeric carbon atoms of the glucose residues at C_3 and C_{12}.

3β,12β,25-Tri(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranoxyloxy)-20(S),24(R)-epoxydammarane (VII). mp 153-155°C (ethanol); $[\alpha]_D^{20}$ —8.3° (c 1.0; chloroform).

PMR spectrum (δ ppm): 0.74-1.14 (3 H × 8, s) — protons of methyl groups; 2.01-2.10 (3 H × 12, s) — protons of acetyl groups; 3.68 (1 H, m) — proton at C_{24}; 5.06 (1 H, d, $J_{1,2}$ = 7 Hz), 4.56 (1 H, d, J = 7.5 Hz), 4.46 (1 H, d, $J_{1,2}$ = 7.5 Hz) — protons at anomeric carbon atoms of glucose residues.

General Procedure for Saponifying the Acetylated Glycosides

Glycoside (V) was deacylated with a 10% solution of KOH in methanol, and glycosides (VI) and (VII) with a 0.1 N solution of sodium methanolate in methanol. The completeness of the acetylation was checked with the aid of TLC in system 2. The solution was neutralized with KU-2 resin (H^+ form), and was evaporated. The yields of free glycosides amounted to 90-95%.

12β,25-Di-O-β-D-glucopyranosyloxy-20(S),24(R)-epoxydammaran-3α-ol (VIII). $[\alpha]_D^{20}$ −21.2° (c 0.1; pyridine), $C_{42}H_{72}O_{14}$.

3α,12β,25-Tri-β-D-glucopyranosyloxy-20(S),24(R)-epoxydammarane (X). $[\alpha]_D^{20}$ −21.4° (c 0.8; pyridine), $C_{48}H_{82}O_{19}\cdot 3H_2O$.

3β-12β,25-Tri-β-D-glucopyranosyloxy-20(S),24(R)-epoxydammarane (XI). $[\alpha]_D^{20}$ −20.8 (c 1.0; pyridine), $C_{48}H_{82}O_{19}\cdot 3H_2O$.

SUMMARY

The 12,25-di-O- and 3,12,25-tri-O-glucosides of 20(S),24(R)-epoxydammarane-3α,12β,25-triol and of its 3-epimer have been obtained under the conditions of the orthoester method of glycosilation and by Helferich's modification.

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ANALYSIS OF AN EXTRACT OF *Atropa belladonna* BY CENTRIFUGAL COLUMN CHROMATOGRAPHY

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The possibility has been shown of using the method of centrifugal column chromatography to accelerate the process of separating medicinal substances in mixtures.

The development of methods for analyzing mixtures of medicinal substances is associated with the development of fast, simpler, and improved methods of separating the initial mixtures. One of such methods is centrifugal column chromatography. In this process, the speed of separation of multicomponent medicinal preparations depends on the rate of passage of the extractant through the column. The separation can be accelerated by feeding the extractant to the column under a pressure created with the aid of complex and expensive pumping systems or with the aid of centrifugal forces.

It must be mentioned that the method of separation on columns under the action of centrifugal forces has not been used to solve the problems with which pharmaceutical analysis is faced. The method developed has previously been used for separation with the subsequent determination of steroid hormones [1], sterols and fatty acids [2], amino acids [3], pigments [4], carbohydrates [5], blood components [6], anthraquinone derivatives [7], etc. Investigations performed have shown undoubted advantages of centrifugal column chromatography in comparison with ordinary column chromatography, such as speed of analysis, small amounts of the initial substances and reagents required, and simplicity of the apparatus used.

The separation of medicinal substances in mixtures is carried out in centrifugal fields on a TsLK-1 centrifuge having three strictly fixed speeds: 1000, 1500, and 3000 revolutions per minute. In view of the fact that under the action of a high centrifugal force creating a pressure above 100 atm some adsorbents undergo extreme self-compaction, we have created a scheme using a laboratory autotransformer. The autotransformer ensures a variation in the

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