THE STRUCTURE OF CALENDULOSIDE A

L. P. Vecherko, É. P. Zinkevich, N. I. Libizov, and A. I. Ban'kovskii

We have previously [1] isolated a mixture of eight glycosides of oleanolic acid from the roots of Calendula officinalis L. and have proposed a partial structure for the least polar glycoside — calenduloside A (II). In the present paper we give the results of the isolation and the determination of the complete structure of (II) and also of a minor component of the mixture of glycosides — a monoside of oleanolic acid (I), which we obtained also by the incomplete acid hydrolysis of (II).

The monoside (I), with the composition C_{36}H_{58}O_{8}, which is present in the roots of C. officinalis in the form of traces was detected in small amounts when the combined glycosides were chromatographed on a column of silica gel and was also isolated from the products of the incomplete hydrolysis of this mixture (in the latter case, the yield was 7.5%). The identity of the samples was confirmed by the absence of a depression of the melting point of a mixture and by their IR spectra.

Calenduloside A (II), C_{42}H_{68}O_{13} • H_{2}O, was obtained by chromatographing the combined glycosides on a column of silica gel; yield 3%. The two glycosides give a tetraacetate C_{44}H_{66}O_{12} and a heptaacetate C_{56}H_{82}O_{20}, respectively, and the permethylates, C_{41}H_{68}O_{8} and C_{50}H_{84}O_{13}.

According to the UV spectrum, glycosides (I) and (II) contain a trisubstituted olefinic group (\text{max} 204-206 \text{ nm}, \epsilon 4514), while the IR spectra show the presence of a free carboxy group (1700 cm^{-1}) and a hydroxy group (3440 cm^{-1}).

The presence of a carboxy group is also confirmed by the solubility of (I) and (II) in alkalis (they are practically insoluble in water), by potentiometric titration, and by the formation of methyl oleanolate on hydrolysis of the permethylates.

When (I) and (II) were subjected to complete hydrolytic decomposition, in addition to oleanolic acid the hydrolysate was found to contain D-glucose in the case of (I), and D-glucose and D-galactose in the case of (II).

The partial hydrolysis of (II) by dilute mineral acids and 10\% oxalic acid led to a mixture of D-galactose, D-glucose, the monoside (I), and oleanolic acid. No disaccharide (lactose) was found in the products. On the acetolysis of (II) and subsequent diacetylation [2], lactose was identified by PC and TLC.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mol. wt.</th>
<th>(\alpha_{D}^{20}) (methanol)</th>
<th>(\beta_{D}^{20}) (methanol)</th>
<th>(\alpha)</th>
<th>Form of bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl (\alpha)-D-glucopyranoside</td>
<td>194,2</td>
<td>-149</td>
<td>289,4</td>
<td>+141</td>
<td></td>
</tr>
<tr>
<td>Methyl (\beta)-D-glucopyranoside</td>
<td>194,2</td>
<td>-25</td>
<td>-48,6</td>
<td>312,7</td>
<td></td>
</tr>
<tr>
<td>Methyl (\alpha)-D-galactopyranoside</td>
<td>799.0</td>
<td>-41,4</td>
<td>330,8</td>
<td>+21,5</td>
<td>(\beta)</td>
</tr>
<tr>
<td>Calenduloside A</td>
<td>618.86</td>
<td>55,7</td>
<td>329,3</td>
<td>+1,5</td>
<td>(\beta)</td>
</tr>
<tr>
<td>The monoside</td>
<td>456.7</td>
<td>+80.0</td>
<td>+365,3</td>
<td>-36</td>
<td>(\beta)</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1

The difficulty of the hydrolysis with dilute mineral acids [3] shows that the sugars have the pyranose form, which is also confirmed by the formation of 2,3,4,6-tetra-O-methyl-D-glucopyranose in the hydrolysis of the permethylate of (I) and of 2,3,6-tri-O-methyl-D-glucopyranose and 2,3,4,6-tetra-O-methyl-D-galactopyranose in the hydrolysis of the permethylate of (II).

Methylation was effected with methyl iodide in the presence of sodium hydride in dimethyl sulfoxide [4, 5].

The configurations of the glycosidic centers were established from the difference in the molecular rotations between the bioside (II) and the monoside (I), and between the monoside (I) and oleanolic acid in accordance with Klyne's rule [6] (Table 1), by comparison with the molecular rotations of methyl α- and β-D-glucosides and α- and β-D-galactosides given in the literature [7].

On the basis of the information given, the complete structures of the monoside (I) and of calenduloside A (II) can be represented in the following way:

The 3-O-β-D-glucopyranoside of oleanolic acid in the form of the hydrate \( \text{C}_{36}\text{H}_{58}\text{O}_{8} \cdot 2\text{H}_{2}\text{O} \) has been obtained synthetically [8, 9] and has recently been found in Beta vulgaris [10].

EXPERIMENTAL

Chromatography was carried out on type KSK silica gel and type M ("slow") paper of the Leningrad Volodarskii Mill with the following systems of solvents: 1) chloroform-methanol-water (61:32:7); 2) chloroform-methanol (9:1); 3) butan-1-ol-pyridine-water (6:4:3); 4) benzene-butanol-1-ol-pyridine-water (1:5:3:3); 5) ethyl acetate-n-propanol-water (2:7:1); 6) chloroform-ethanol (25:1); 7) chloroform-ethyl acetate (10:1); 8) butan-1-ol-ethanol-water (5:1:4); 9) benzene-acetone-water (5:5:1); 10) chloroform-acetone (10:1); 11) chloroform-methanol-water (10:2:3); 12) chloroform-acetone (4:1); and, 13) chloroform-methanol (7:3).

The sugars were revealed by aniline phthalate and a mixture of aniline, diphenylamine, and phosphoric acid, and the glycosides and their derivatives with 20% sulfuric acid.

The IR spectra of the substances were taken on a UR-10 spectrophotometer (paraffin oil) and the UV spectra on a "Hitachi" recording spectrophotometer. The potentiometric titration was performed on a LP-58 pH-meter.

The gas-liquid chromatography was carried out on a "Pye" chromatograph (column filled with Chromosorb W impregnated with 10% of neopentyl glycol succinate with nitrogen as the carrier gas at 150°C) and a "Chrom 2" instrument [Chromosorb W impregnated with 5% of poly(neopentyl glycol succinate) with argon as the carrier gas at 169°C].

The NMR spectra were recorded on a Varian HA-100 spectrometer (with deuteropyridine as the solvent and tetramethylsilane as internal standard).

The melting points were determined on a Kofler block. The analytical figures for all the compounds corresponded to those calculated.

Isolation of the Glycosides. The comminuted and chloroform-defatted roots of C. officinalis (4 kg) were exhaustively extracted with methanol. The concentrated methanolic extracts deposited a precipitate which was filtered off and washed with acetone. Yield 100 g. According to TLC on silica gel in system 1, the mixture obtained consisted of eight glycosides.