COMPACTIN – A NEW OROBOL BIOSIDE FROM Genista compacta

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From the epigeal part of Genista compacta has been isolated the new isoflavone bioside compactin – $C_{27}H_{30}O_{16}$, mol. wt 610, mp 206-208°C, $[\alpha]_D^{20}$ +35.8° – for which on the baseos chemical transformations and spectral characteristics the structure of 7-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyransyloxy]-3',4', 5-trihydroxyisoflavone, or orobol 7-O-β-sophoroside, has been established.

Studying the composition of benzo-γ-pyrone derivatives from the epigeal part of Genista compacta Schischk. collected in May, 1982, in the region of the village of Podkumok, Savropol krai, in addition to the known compounds genistein, 5-methylgenistein, luteolin 7-glycoside, and ononin [1], we have isolated a new isoflavone glycoside.

The flavonoids were isolated by extracting the air-dry material with 50-60% ethanol after its preliminary treatment with liquid carbon dioxide or a superhigh-frequency (SHF) dielectric treatment. The use of these methods enables the yield of benzo-γ-pyrone derivatives to be increased with a simultaneous saving of time and of solvents in the subsequent purification of the extract. After the evaporation of the extract and the addition of chloroform, a compound having the composition of $C_{27}H_{30}O_{16}$, mp 206-208°C separated out at the chloroform–water boundary.

Substance A gave positive reactions with solutions of ferric chloride and alkali but did not take part in the azo-coupling reaction with a freshly prepared solution of diazotized sulfanilamide [2], which is characteristic for chromone derivatives having a free hydroxy group at C_7. The UV spectrum of an ethanolic solution of the substance showed a single intense absorption maximum at 262 nm. The form of the curve was characteristic for an isoflavone [3]. The absence of a bathochromic shift in the presence of freshly fused sodium acetate confirmed that the chromone nucleus lacked a free OH group at C_7. A bathochromic shift of the absorption maximum on the addition of aluminum chloride [3] and the appearance of an olive coloration of the solution of the substance under investigation with ferric chloride indicated the presence of a free hydroxy group at C_5. This substance also gave a bathochromic shift of the absorption maximum in the presence of boric acid and sodium acetate, which is characteristic for flavonoids having an ortho-dihydroxy grouping in the lateral phenyl radical [3]. The UV-spectral characteristics of the compound isolated from G. compacta and its aglycone are given below:

	C_2H_5OH	C_2H_5ONa	$CH_3COONa + H_3BO_3$	CH_3COONa	$AlCl_3$	$AlCl_3+HCl$
Substance A	262, 296 sh; 340 sh.	272, 307 sh.; 337 sh.	271, 353 sh.	263, 340 sh.	274, 383 sh.	272, 363 sh.; 383
Aglycone of substance A	263, 294 sh.	270, 339 sh.	268, 295 sh.	269, 330 sh.	275, 374 sh.	273, 374 sh.

The IR spectrum of the substance showed characteristic absorption bands of the carbonyl group of a γ-pyrone (1660 cm^{-1}) of the π-conjugation of an aromatic system (1615, 1565 cm^{-1}), of hydroxy groups (3380-3370 cm^{-1}), and of substituents in the lateral phenyl radical (845, 855 cm^{-1}) [4].

The PMR spectrum contained the signals of a carbohydrate component (3.92-4.64 ppm; 10 H) and the signals of aromatic protons in the nucleus and in the lateral phenyl radical (6.52, 6.87, 7.21, 7.58 ppm, 6 H). A singlet at 7.21 indicated the isoflavone nature of the substance (signal of the H-2 atom of the isoflavone nucleus) [3]. A multiplet at 5.53 ppm with J = 6 Hz showed that substance A was a 7-O-β-glycoside [3].

The glycosidic nature of the substance was confirmed by acid hydrolysis, which gave an

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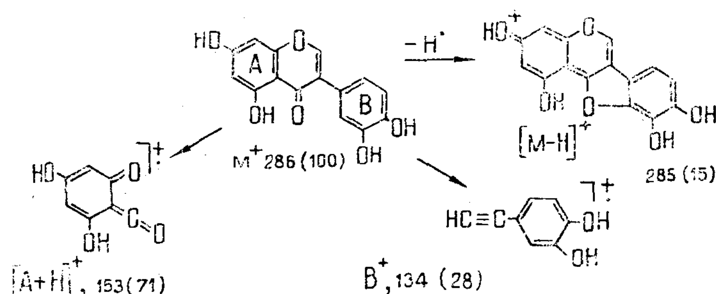
aglycone with the composition $C_{15}H_{10}O_6$, mp 270°C. According to the results of UV spectroscopy, it had free OH groups at C_5, C_3, and C_4, and, in contrast to substance A, also at C_7. The IR spectra showed the absorption bands of a carbonyl group (1665 cm^{-1}), of the π-congugation of aromatic rings (1620, 1565 cm^{-1}), of substitution in the lateral phenyl radical (870, 810, 745 cm^{-1}), and of OH groups (3350-3380 cm^{-1}) [4].

The PMR spectrum had the signals of the H-6, H-8, H-2', H-5', and H-6' protons (5 H), and also the signal of the H-2 proton (7.63 ppm, s).

The mass spectra of the aglycone showed the peak of the molecular ion M^+ with m/z 286 (100%), and the peaks of ions with m/z 285, 281, 270, 243, 231, 229, 153, and 134. It is known that the presence of an intense M − 1 ion is characteristic for simple isoflanones and that the fragmentation of the molecular ion takes place in the same way as for flavones [5]. In view of this, we assumed that the following fragmentation took place on electron impact:



Strong peaks of ions with m/z 153 and 134 showed that there were four OH groups in the aglycone. This was also shown by the formation of phloroglucinol and of homoprotocatechuic acid when the aglycone was fused with alklai.

Thus, it may be connected that the aglycone of substance A was 3',4',5,7-tetrahydroxyisoflavone, which is known under the name of orobol.

D-glucose was found in the hydrolysate after substance A had been boiled with 10% sulfuric acid for 6 hours. The yield of the aglycone (46%) showed that substance A contained two D-glucose residues. The results of enzymatic hydrolysis with purified emulsin indicated the β-configuration of the glycosidic bond. Acid hydrolysis under mild conditions led to the formation of D-glucose and a biose. In order to establish the structure of the biose we took gentiobiose, cellobiose, and sophorose for comparison. We isolated the last-mentioned compound from kaempferol 3-β-sophoroside which one of us had obtained previously from the fruit of the Japanese pagoda tree (*Sophora japonica* L.) [6]. In addition, the carbohydrate component was identified by the methylation of substance A and of kaempferol 3-β-sophoroside with dimethyl sulfate in the presence of 10% KOH followed by hydrolysis of the reaction products obtained and a comparison of the methylated carbohydrate residues by chromatographic methods. It was concluded from its chromatographic behavior that the carbohydrate component in substance A was sophorose: O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranose.

On the basis of the results obtained, we can characterize substance A as 7-[O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyloxyl-3',4',5-isoflavone, or orobol 7-β-D-sophoroside.

The follwoing glycosides of orobol are known: orobol 7-O-β-glucoside, orobol 7-O-rhamnoglycoside, orobol 8-C-glucoside, and orobol 6,8-di-C-glucoside [7]. We have found in the literature no orobol glycosides similar in structure to that described, and we have given it the name of compaction.

EXPERIMENTAL

Melting points were determined on a Kofler block, and specific rotations on a Polamat A polarimeter. Elementary analysis was performed on a Hewlett-Packard 158B automatic CHN analyzer. The spectral characteristics were obtained on SF-4A (UV) and UR-20 (IR) spectrophotometers, on a Varian HA-100 spectrometer at 100 MHz (PMR) and Varian CH-8 mass spectrometer at 70 eV. Chromatographic analysis was performed by TLC (Silufol plates in systems: 1) BAW (4:4:7) and 2) BAW (9:11:10)) and by PC (Filtrak FN-15 in systems 3) BAW (4:1:5) and

4) 15% AcOH).

Isolation of the Compound. The air-dry raw material (657 g) was first treated with liquid carbon dioxide (20-22°C, 5.8-6.18 MPa, 80 min) or was subjected to SHF heating (Slavyanka furnace, 2.2 kW, 2375 MHz, 150 sec), after which it was extracted with 50-60% ethanol on the water bath under reflux (80°C, 3 h). The ethanolic extract was evaporated and was purified with chloroform. A light grey precipitate separated out at the chloroform–water boundary. It was purified by fractional crystallization from aqueous ethanol (yield 877 mg).

Substance A formed a light grey powder with mp 206-208°C, $[\alpha]_D^{20}$ +35.8° (c 1.0; CH_3OH), R_f value in system 1 – 0.83; 2 – 0.85; 3 – 0.23; 4 – 0.67. UV spectrum (ethanol), λ_{max}, nm: 340 sh,., 296 sh., 2.62. IR spectrum (KBr), cm^{-1}:3380–3370, 1660, 1630, 1615, 1565, 1480, 1100, 920, 885, 845, 800, 760, 740. PMR spectrum ($[^2H_6]$Py, TMS, δ scale, ppm): singlets of six H aromatic rings – 7.58, s; 7.21, s; 6.87, d; 6.52, d; of the anomeric proton of a glucose residue – 5.53, m, J = 6 Hz; and 10 H of the carbohydrate component – 3.92-4.64 m.

The Acid Hydrolysis of Substance A. A solution of 0.1 g of the glycoside in 20 ml of ethanol was treated with an equal volume of 10% H_2SO_4 solution. The mixture was boiled in an air bath in a flask with a reflux condenser for 6 h. D-Glucose was detected in the hydrolysate and the aglycone (in a yield of 0.046 g) in the precipitate. B. A mixture of 0.05 g of the glucoside and 10 ml of 2% H_2SO_4 was boiled in the air bath under reflux. The course of hydrolysis was monitored with the aid of TLC every 20 min. Sophorose, D-glucose, and the aglycone were detected in the hydrolysate.

Enzymatic Hydrolysis of Substance A. A solution of the glycoside (0.01 g) in water (20 ml) was cooled and was treated with 2 ml of a colloidal solution of emulsion (0.01 g in 10 ml of water), after which the mixture was left in a thermostat at 36°C for 24 h. The hydrolysis proudcts were diluted with 96% ethanol (1:1) and the resulting mixture was heated to the boil. Sophorose was detected in the hydrolysate.

Methylation of Substance A. A mixture of 0.5 g of the glycoside and 5 ml of water was treated with 3 ml of dimethyl sulfate and 4 ml of 10% KOH, and the resulting mixture was shaken for 32 h. In the first 6 h, 1-ml protions of 10% KOH were added every 30 min. After the end of the reaction (monitoring by TLC), hydrolysis was performed with 10% H_2SO_4 in the air bath in a flask with a reflux condenser (4 h). After cooling (24 h) the precipitate that had deposited was separated off. In the remaining hydrolysate, after neutralization (pH 5.5), methylated carbohydrate residues were identified by PC and TLC.

The aglycone of substance A formed colorless crystals with mp 270°C; R_f in system 3 – 0.80; 4 – 0.54. UV spectrum (ethanol) λ_{max}, nm: 338 sh., 294 sh., 263. IR spectrum (KBr), cm^{-1}: 3385, 3350, 1665, 1620, 1565, 1470, 870, 810, 745.

PMR spectrum ($[^2H_6]$Py, TMS, δ-scale), ppm: 7.63 (H-2); 7.28, 6.87, 6.49 (H-2', H-5', H-6', H-8, H-6). Mass spectrum, m/z, %: 286 (100), 285 (15), 281 (12), 270 (28), 243 (9), 231 (17), 229 (9), 219 (15), 181 (36), 169 (40), 162 (5), 153 (71), 134 (28), 131 (55), 119 (67).

Alkaline Fusion of the Aglycone. A mixture of 0.01 g of the substance and 5 g of caustic potash was fused in a sealed tube for 2 min. After cooling, water was added, and the solution was neutralized with 10% H_2SO_4 and extracted with 50 ml of diethyl ether. The reaction products were identified by PC in the presence of markers.

SUMMARY

From the epigeal part of Genista compacta Schischk. the new isoflavone glycoside compaction has been isolated, and its structure has been established as 7-[O-β-D-glucopyranosyl-(1→2)- O-β-glucopyranosyloxyl-3'4',5-trihydroxyisoflavone or orobol 7-β-sophoroside.

LITERATURE CITED

1. L. K. Klyshev, V. A. Bandyukova, and L. S. Alyukina, Plant Flavonoids [in Russian], Alma-Ata (1978), p. 220.
2. V. A. Bandyukova, Rast. Resur., No. 4, 591 (1965).
3. T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavones, Springer, New York (1970), p. 354.
4. H. Wagner, in: Methods in Polyphenol Chemistry, Proceedings of the Plant Phenol Group. Symposium, Oxford, (1964), p. 37.

5. N. Morita and M. Arisawa, Heterocycles, 4, No. 2, 373 (1976).
6. V. A. Bandyukova, in: The Study and Use of the Plant Resources of the USSR [in Russian], Moscow (1964), p. 209.
7. J. L. Ingham, Fortschr. Org. Naturstoffe, 266 (1983).

THE COMPOSITION OF THE ESSENTIAL OILS OF Thymus marschallianus

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The essential oil of Thymus marschallianus was obtained by steam distillation of freshly-gathered flowering plants growing at various heights of the Trans-Ili Ala-Tau range (Kazakhstan). The number of components detected was 49, of which 35 were identified. No appreciable differences in the composition of the essential oil with a change in the height of growth of the plant were observed. The essential oil of Th. marschallianus and of a number of species growing in various countries have been compared.

Some species of thyme are widely used in folk medical practice as sudorific and diuretic agents and sedatives in diseases of the respiratory tracts, and also in the form of lotions and ointments in acute rheumatism and various skin diseases [1]. In scientific medicine, extracts of common thyme and wild thyme are used for the preparatin of pertussin. They are cultivated for this purpose in the Ukraine in Krasnodar krai, and in Bashkir.

In Kazakhstan, Thymus marschallianus Willd. grows on the steppe slopes of the hilly area and the foothills before the forest zone of the mountains, on the gravels of old river beds that are now steppe formations, and on heathergrass—sheep's fescue steppes [2]. In order to study the possibility of the practical use of this species and to determine its position in the genus, we have investigated two samples of the essential oil from populations growing in the foothills of the Trans-Ili Ala-Tau.

Preliminary GLC analysis revealed no differences in the qualitative component compositions of the essential oils of the two populations. The quantitative ratios of the components in the samples of oils differed little and they were therefore investigated together. On a chromatogram of the hydrocarbon fraction of the essential oil, not less than 24 substances were counted (Fig. 1). Terpene hydrocarbons were represented mainly by p-cymene, γ-terpinene, and α-pinene.

A comparison of the IR spectra and retention times of the sesquiterpene hydrocarbons with authentic samples permitted the identification of α-gurjunene, β-elemene, β-caryophyllene, himachalene, β-humulene, γ-cadinene, ar-curcumene, and β-cadinene.

The substance responsible for peak 23 could not be identified in this way. The constants of this hydrocarbon were n_D^{20} 1,4912; d_{20}^{20} 0,8745; $[\alpha]_D^{20}$ −76°. Its molecular weight was 204. According to its IR and mass spectra it contained an isopropylidene group (band at 1380 cm^{-1}; M − 43), a trisubstituted >C = CH— double bond (810, 1670, 3058 cm^{-1}), and a >C = CH_2 methylene double bond (890, 1635, 3080 cm^{-1}). On hydrogenation over Adams platinum oxide in glacial acetic acid, three moles of hydrogen were added with the formation of a hydrocarbon having n_D^{20} 1.4553, with the IR spectrum of bisabolane. Consequently, one of the main components of Th. marschallianus is β-bisabolene, although the constants obtained for it differed somewhat from those given in the literature: d_4^{20} 0,8716, n 1,4912; $[\alpha]_D^{20}$ − 54° [6].

The oxygen containing compounds of the essential oil of Th. marschallianus (Fig. 2) were well separated on programmed columns with an ester phase.

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