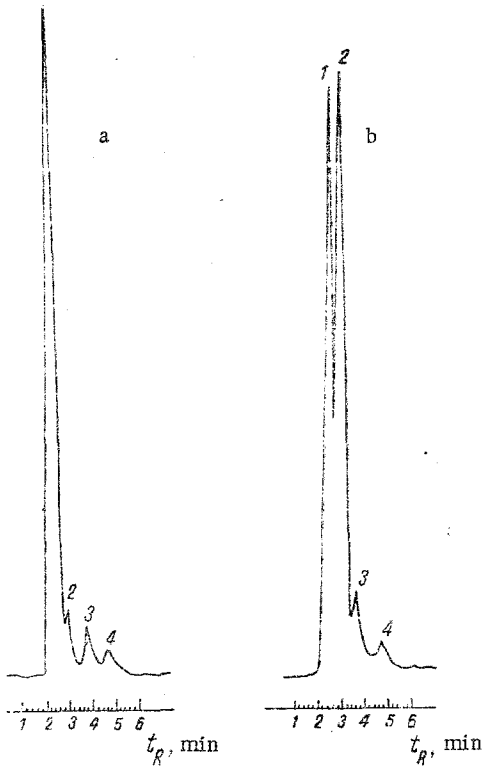

Fig. 4. Separation of the flavonoids of *Datisca cannabina* by liquid chromatography: 1) datiscin; 2) datiscanin (a); after the addition of authentic datiscanin to the initial mixture (b); 3) galanginoside [1]; 4) datinoside [1].

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FLAVONOIDS OF *Datisca cannibina*.

VI. PROPERTIES OF DATISCIN

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UDC 547.972

An authentic individual sample of datiscin has been obtained, its physicochemical constants have been determined, and additional information concerning its structure as 2',3,5,7-tetrahydroxyflavone 3-O-[6"-(O-α-L-rhamnopyranosyl)-β-D-glucopyranoside] has been obtained.

Datiscin (I) was isolated from *Datisca cannabina* as early as 1816 [1]. Until the present time it was the only known glucoside of datiscetin (2',3,5,7-tetrahydroxyflavone) (II), which is present in the same plant [2]. There is contradictory information in the literature relative to the structure of the carbohydrate moiety of (I) [1, 3-8]. Chronologically, the sugar was first identified as glucose [3], then as rhamnose [4], again as glucose [5], and finally as rutinose [6].

In two papers [9, 10], datiscin is characterized as datiscetin 3-rutinoside, although no constants apart from details of the UV spectrum are given. However, in a more recent monograph [11] it is again called datiscetin rhamnoside. In the handbook literature [7, 8],

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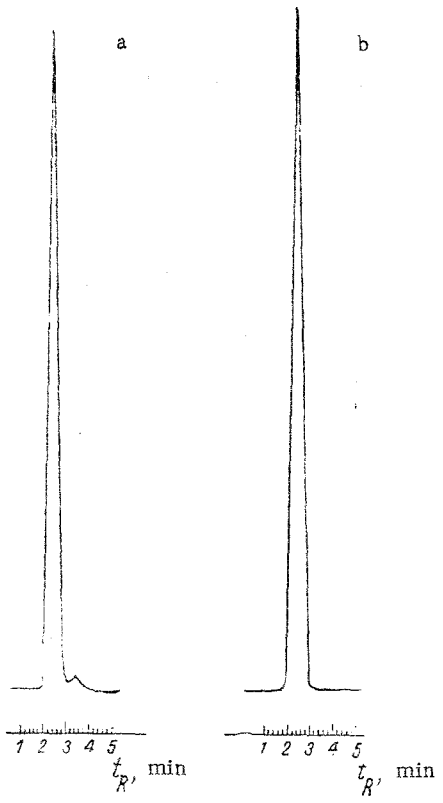


Fig. 1. Check of the purity of datiscin by high-performance liquid chromatography under the conditions given in the previous paper [15].

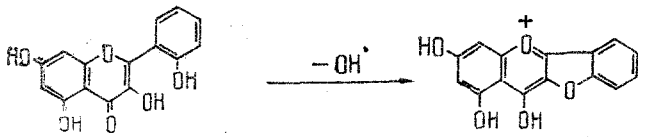
the constants from a 1935 paper by Charaux [6] are given, according to which datiscetin has the formula $C_{27}H_{30}O_{15}$ and crystallizes with four molecules of water; it melts at 192-193°C; $[\alpha]_D = -48.59°$ (c 0.9; ethanol), calculated to the anhydrous substance.

We have confirmed the structure of datiscin as datiscetin 3-rutinoside and have determined its constants more accurately [2, 12]. In the present communication we give some additional information on its properties.

Charaux [6] was probably not dealing with pure datiscin, since he used only recrystallization for its purification. We have shown that the roots and herbage contain a mixture of flavonoids which it is impossible to separate by recrystallization without the use of chromatography, since the glycosidic fraction contains five 3-rutinosides of related flavonols (datiscetin, datin, galangin, izalpinin, and quercetin [2, 13, 4]). To obtain pure datiscin we used chromatography on polyamide, and even in this case came up against difficulties in obtaining a sufficiently pure compound. Thus, narrow fractions obtained from the column and containing, according to TLC results, only one component — datiscin — on being checked by high-performance liquid chromatography were found to contain small amounts of other flavonoid compounds (Fig. 1a). By repeated chromatography we succeeded in obtaining pure datiscin (Fig. 1b), and its constants are given in the present paper.

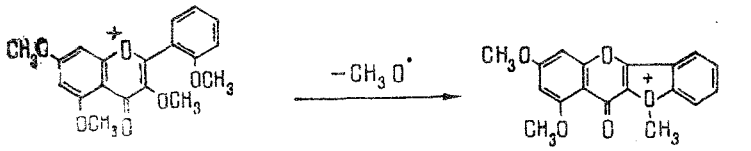
The specific rotation of the datiscin that we obtained was higher than that given in the literature, and the melting point lower (see Experimental). The characteristics of the UV spectrum also differ somewhat. It must be mentioned that datiscin is thermally unstable: the TLC monitoring of the course of heating a sample on a Kofler block showed that at 130°C traces of datiscanin [15] appear, and at 173°C datiscetin is formed; after melting the amount of the aglycone is about 50%. Datiscanin is still less stable. The aglycone from it appears at a temperature as low as 100°C; after melting, it decomposes completely and at 185°C crystals of the aglycone appear which melt at 245-248°C.

The position of the carbohydrate residue in datiscin was confirmed additionally by mass-spectrometric investigations of its aglycone datiscetin (II), and also the tetra- and trimethyldatiscetin. The mass spectrum of (II) contained, in addition to the molecular ion and the ions of the retrodiene reaction (A + H, C), the strong peak of the M — 17 ion, which is characteristic for the fragmentation of 2'-hydroxyflavonols [16]:



M^+ 286 (98%) $(M-OH)^+$ 269 (100%)

This type of fragmentation is also retained for tetramethyldatiscetin:



M^+ 342 (96%) $(M-CH_3O)^+$ 311 (100%)

The dominating peak is the M − CH_3O ion also in the mass spectrum of 2',5,7-trimethyldatiscetin obtained by methylation of datiscin followed by acid hydrolysis. A comparison of the resonance of the methoxy group in deuterochloroform and deuterobenzene [17] for the tri- and tetramethyl ethers of datiscetin demonstrates the presence of a free 3-OH group in trimethyldatiscetin and, consequently, 3-glycosylation in datiscin.

An additional proof of the structure of biose residue is our isolation of the 3-monoglucoside of datiscetin — datiscanin — as the result of stepwise hydrolysis of datiscin [15].

EXPERIMENTAL

For general information, see the preceding paper [15].

The separation of the total flavonoids isolated from the herbage of *Datisca cannabina* [13] was performed by repeated chromatography on columns of polyamide sorbent using as eluent chloroform–methanol–methyl ethyl ketone (12:2:1). The fractions of chromatographically pure datiscin were recrystallized from water and aqueous ethanol. Datiscin crystallizes from diluted solutions in the form of thin lustrous needles.

Datiscin (I). Slightly yellowish thin needles with a greenish tinge and a silky luster, soluble in alcohols and boiling water, and practically insoluble in cold water and acetone. Composition $C_{27}H_{30}O_{15}$, $[\alpha]_D^{20}$ − 63.7° (c 0.9; methanol), mp 175-177°C (decomp.). Maxima in the UV spectra (nm): MeOH — 260 (log ε 4.39); 304 (3.99); 330 infl. (3.95); NaOMe — 268, 325, 368; NaOAc — 260, 350; NaOAc + H_3BO_3 — 261, 306, 335 infl.; $AlCl_3$ and $AlCl_3$ + HCl — 269, 320, 386.

Air-dry datiscin contains 11.7% of water, which it readily loses in a vacuum desiccator over $CaCl_2$ (after 20 h, according to a Karl Fischer determination, the moisture content was 1.5%). Datiscin dehydrates completely in a vacuum desiccator over P_2O_5, but on being kept in air for 2-4 days it rehydrates gradually to a moisture content of 10.8%, which corresponds to the hydrated crystal $C_{27}H_{30}O_{15}\cdot 4H_2O$ and is close to the moisture content of the air-dry product recrystallized from water. Its specific rotation $[\alpha]_D^{20}$ = −58.2° (calculated on the anhydrous substance, −64.7°, c 0.6; ethanol).

PMR spectrum of the TMS ether of (I) in CCl_4 (ppm): 7.6-6.7 (m, H-3',4',5',6'); 6.33 (d, 2 Hz, H-8); 6.11 (d, 2 Hz, H-6); 5.77 (d, 8 Hz, H-1''); 4.27 (d, 2 Hz, H-1'''); 3.9-3.1 (10 H of rutinose); 0.87 (d, 6 Hz, CH_3 of rhamnose). PMR spectrum of (I) in CD_3OD at 20°C (ppm): 7.6-6.8 (m, 4 H); 6.34 (d, H-8); 6.22 (d, H-6); 5.03 (d, 7 Hz, H-1''); 4.64 (d, 2 Hz, H-1'''); 3.9-3.1 (10 H of rutinose); 1.2 (d, 6 Hz, CH_3). The signal of the anomeric proton of the glucose residue (δ 5.05) is masked by the signals of the mobile protons at 20°C but can be seen at 60°C. Both doublets of the anomeric protons (δ 5.03 and 4.64) can readily be seen in acidified CD_3OD, where the signals of the mobile protons are shifted downfield.

Datiscetin (II). Yellowish crystals with mp 276-278°C (decomp.); composition $C_{15}H_{10}O_6$. Maxima in the UV spectrum (nm): MeOH — 260 (log ε 4.28), 308 (3.93), 350 (4.04); NaOMe — 277, 328, 390; NaOAc — 273, 395; NaOAc + H_3BO_3 — 265 , 376; $AlCl_3$ and $AlCl_3$ + HCl — 268, 333, 410. PMR spectrum of the TMS ether in CCl_4 (ppm): 7.4-6.7 (m, 4 H); 6.3 (d, H-8); 6.05 (d, H-6). Mass spectrum: M^+ 286 (98%), M − OH 269 (100), A + H 153 (43), C 121 (13).

Tetraacetate, mp 143-145°C (from ethanol).

Tetramethyldatiscetin, $C_{19}H_{18}O_6$, mp 151-152°C. Fragment of the PMR spectrum in $CDCl_3$ (singlets of four CH_3O groups); 3.9 (3 H); 3.76 (6 H); 3.74 (3 H); in C_6D_6 — 3.23 (3 H); 3.30 (3 H); 3.40 (3 H); 3.88 (CH_3O at C-3). Mass spectrum: M^+ 342 (96%), M — H 341 (61), M — CH_3 327 (13), M — CH_3O 311 (100), M — CH_3CO 299 (31), A + H 181 (25), C 135 (6).

Methylation and Acid Hydrolysis of (I). The methylation of datiscin with dimethyl sulfate followed by acid hydrolysis under the conditions given previously [18] yielded 2',5,7-trimethyldatiscetin with the composition $C_{18}H_{16}O_6$, mp 148-149°C (from ethanol). Maxima in the UV spectrum, (nm): MeOH — 247, 297, 336; NaOME — 256, 366; NaOAc — 247, 297, 336; $AlCl_3$ and $AlCl_3$ + HCl — 259, 388. Fragment of the PMR spectrum in $CDCl_3$, singlets of three CH_3O groups: 3.9 (3 H); 3.76 (6 H); in C_6D_6; 3.23 (3 H); 3.40 (3 H); 3.43 (3 H). Mass spectrum, m/z (intensity, %): M^+ 328 (100), M — CH_3 313 (3), M — CH_3O 297 (70), A + H 181 (35), C 135 (25).

Acetylation of (I). The acetylation of datiscin with acetic anhydride in pyridine led to the nonaacetate — colorless crystals with mp 112-116°C, $[\alpha]_D^{20}$ −60.6° (c 0.7; acetone). PMR spectrum in CCl_4 (ppm): 7.8-7.2 (m, H-3',4',5',6'); 7.15 (d, H-6); 6.75 (d, H-8); 5.2-4.4 (m, 8 H of rutinose); 3.7-3.0 (m, 4 H of rutinose); 2.37 (s, 3 H); 2.26 (s, 3 H); 2.17 (s, 3 H); 2.1 (s, 6 H); 1.95 (s, 9 H); 1.86 (s, 3 H); 1.0 (d, 6 Hz, CH_3 of rhamnose).

CONCLUSION

In view of the contradictory and incomplete literature information on datiscin, an authentic pure sample of datiscin has been obtained and its physicochemical constants have been determined.

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