ALKYLIDENE DERIVATIVES OF DATISCIN

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With lower ketones (acetone, methyl ethyl ketone) in weakly acidic solution, flavonoid rhamnosides and rutinosides form alkylidene derivatives at the cis-diol grouping of the rhamnose residue. It has been found that the formation of alkylidene derivatives of flavonoid glycosides is a side reaction that takes place during the chromatographic process on an "acid" polyamide sorbent.

In order to improve the sorption properties of a polyamide with respect to certain classes of compounds, some workers [1] modify it by activation with acid or alkali. The "acid" sorbent is obtained by treating a polyamide powder with a 3-5% solution of hydrochloric acid at room temperature for 2-3 h followed by washing with distilled water to neutrality.

In our work with the repeated use of a polyamide sorbent, in order to regenerate it the column was washed successively with acidified methanol (five drops of concentrated HCl to 100 ml), with water, and with methanol. To separate a mixture of flavonoids from the herbage of *Datisca cannabina* [2] chloroform–methanol–methyl ethyl ketone (12:2:1) was used as the eluent. This led to a clear separation of the components cannabin, diatoside, galanginoside, datiscin, and rutin, which are the 3-rutinosides of various flavonols.

In the TLC analysis of the fractions, new substances not present in the initial mixture of flavonoids were detected in each of them. On the basis of the fact the appearance of one substance corresponds to each flavonoid glycoside after passage through the column and, on the whole, the new substances possess greater chromatographic mobilities with the same chemical reaction with the participation of the flavonoid glycosides.

To elucidate the role of the polyamide sorbent and the eluent mixture, we have studied the behavior of one of the flavonoid glycosides – datiscin – both on its passage through a column under the conditions described above and on its dissolution in an acidified mixture of methanol and methyl ethyl ketone, i.e., with the exclusion of the polyamide sorbent. In both cases the datiscin took part in a chemical reaction leading to the formation of substance (I), which was isolated preparatively by chromatography on silica gel (elution with 3% methanol in chloroform yielded (I), while 10% methanol in chloroform gave the initial datiscin).

The NMR spectrum of substance (I) contains, in addition to the signals of datiscin, the signals of CH_3 and CH_3CH_2 groups, and in the region of resonance of the carbohydrate protons of 3-6 ppm two signals are shifted downfield relative to datiscin – the doublet at 4.05 ppm (J = 5 Hz), and the quartet at 3.9 ppm (J_1 = 5, J_2 = 8 Hz). This gave grounds for considering that datiscin had added a molecule of methyl ethyl ketone at the carbohydrate moiety.

When the methyl ethyl ketone was replaced by acetone, substance (II) was obtained, the PMR spectrum of which contained the singlet signals of two CH_3 groups but was otherwise similar to that of substance (I) (Fig. 1). It can be seen from the PMR spectrum of its full acetate (III) that two of the OH groups of rutinose were not acetylated. This gave us grounds for considering that compound (II) was the isopropylidene, and compound (I) the isobutylidene, derivative of datiscin. In order to assign the signals of rutinose shifted downfield in the alkylidene derivative, we compared the PMR spectra of compounds (I), (II), (III), datiscin, and the full acetate of datiscin [3], varying the solvent and the temperature, but no additional facts were revealed. But since it is known [4] that alkylidene derivatives with lower ketones are formed in the case of carbohydrates with the cis configuration of α-hy-

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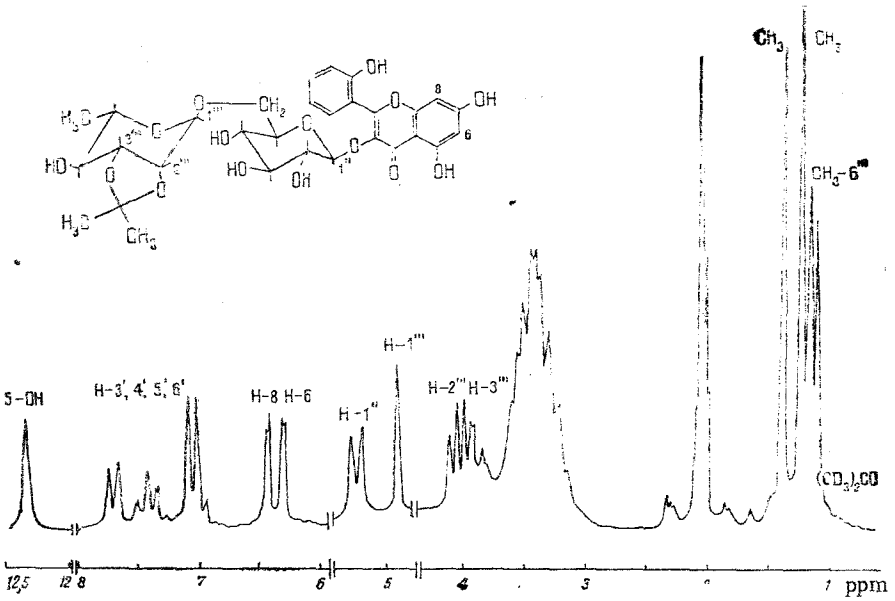

Fig. 1. PMR spectrum of isopropylidenedatiscin (II) in deuteroacetone.

hydroxy groups it is easy to assume that in our case the 2''',3'''-hydroxy groups of the rutinose residue, i.e., the cis-diol grouping of the rhamnose residue, are involved. This hypothesis was confirmed in quercitrin (quercetin 3-O-α-L-rhamnopyranoside), used as a model compound. We obtained isopropylidene quercitrin (IV) and its pentacetate (V) and also the full acetate of quercitrin (VI). In the PMR spectra of (IV), (V), and (VI) it was easy to make an assignment of all the carbohydrate protons, and a comparison of them showed that the rhamnose residues in these compounds give different signals. The coupling constants of the protons in compound (VI) did not contradict the $^1C_4$ conformation of the L-rhamnose residue. It was obvious from a Dreiding model that the closure of the five-membered rings in the formation of the isopropylidene derivatives (V) and (VI) causes a distortion of the chair-like $^1C_4$ conformation, and the measured angles agree well with those calculated by the Karplus equation on the basis of the vicinal coupling constants. In the spectrum of (V), as compared with that of (IV), the signal of the gem-acyl H-4" proton has shifted downfield (in the spectrum of (VI) there is only one signal of an aliphatic acetoxy group, at 2.04 ppm, which can be assigned to 4"-OAc), from which it follows that the 2",3"-OH groups of the rhamnose residue have reacted with the acetone.

Thus, by comparing the PMR spectra of isobutylidenedatiscin (I), isopropylidenedatiscin (II), and isopropylidenequercitrin (IV) it is possible to assert that acetone and methyl ethyl ketone react with the 2''',3'''-dihydroxy grouping of rutinose, i.e., (II) has the structure shown in Fig. 1.

The reaction of flavonoid rutinosides with lower ketones in an acid medium that we have found leads to the conclusion of the necessity for a careful washing of the "acid" polyamide in order to avoid a chemical reaction and, on the other hand, compels a critical analysis of those cases of the isolation of alkylidene derivatives from natural extracts when the extraction process is carried out with acetone in a weakly acid medium the creation of which may be brought about by the organic acids that are present in plants.

EXPERIMENTAL

For general information, see our previous paper [5].

2''',3'''-Isobutylidenedatiscin (I). Datiscin (50 mg) was dissolved in a few drops of methanol, and 50 ml of methyl ethyl ketone and 2 drops of concentrated hydrochloric acid were added. After 2 h, the solution was neutralized by the addition of a few grains of Dowex anion-exchange resin (HCO_3^-) and it was rapidly filtered and the solvent was evaporated off. The residue was deposited on a column of silica gel, which was washed with chloroform, and then 3% of methanol in chloroform eluted compound (I).

Compound (I) has mp 138-142°C; $[\alpha]_D^{20}$ −42.5° [c 0.9 acetone–methanol (1:1). PMR spectrum in deuteroacetone (ppm): 12.3 (s, 5-OH); 7.8-7.0 (m, H-3',4',5',6'); 6.42 (d, 2 Hz, H-8); 6.26 (d, 2 Hz, H-6); 5.2 (d, 8 Hz, H-1"); 4.86 (s, H-1'"); 4.05 (d, 5 Hz, H-2'"); 3.9 (q, 5 and 8 Hz, H-3'"); 3.7-3.9 (8 H of rutinose); 1.6 (q, 8 and 8 Hz, $-\underline{CH_2}CH_3$); 1.22 (s, $-CH_3$); 1.17 (d, 6 Hz, CH_3 of rhamnose), 0.9 (t, 8 Hz, $-CH_2\underline{CH_3}$).

2'",3'"-Isopropylidenedatiscin (II). This was obtained and purified in a similar manner to that described above for compound (I), with the methyl ethyl ketone replaced by acetone.

Compound (II), mp 148-150°C, $[\alpha]_D^{20}$ −66.4° (c 0.6; acetone). The PMR spectrum in deuteroacetone (see Fig. 1) coincided with that given for (I) with the exception of the 0.5-2.0 ppm region: 1.36 (s, CH_3); 1.20 (s, CH_3); 1.12 (d, 6 Hz, CH_3 of rhamnose).

Heptaacetate of (II) and (III). The acetylation of (II) was carried out with acetic anhydride in pyridine at 20°C for 24 h. After the addition of ice water, the amorphous colorless substance was carefully washed with water and dissolved in acetone, the solution was evaporated, and the residue was dried in vacuum over P_2O_5. mp 104-106°C, $[\alpha]_D^{20}$ −33.0° (c 0.7; acetone). The PMR spectrum in CCl_4 contained uninterpretable signals of carbohydrate protons (5.3-4.5, 3.9-3.0 ppm, 12 H), three singlets of aromatic acetoxy groups (2.37, 2.25, 2.15 ppm), four singlets of acetoxy groups in rhamnose (2.06 ppm) and glucose (1.96, 1.92, 1.90 ppm) residues, singlets of isopropylidene CH_3 groups (1.42 and 1.18 ppm), and a doublet of a rhamnose CH_3 (0.92 ppm, 6 Hz).

2",3"-Isopropylidenequercitrin (IV). A solution of 0.2 g of quercitrin in 0.2 ml of methanol was treated with 50 ml of acetone and three drops of concentrated HCl. The subsequent working up procedure and purification were similar to those described for (I).

Compound (IV), mp 115-118°C, $[\alpha]_D^{20}$ − 76.2° (c 0.07; acetone). PMR spectrum in deuteroacetone (ppm): 7.5 (d, 2 Hz, H-2'); 7.4 (q, 2 and 8 Hz, H-6'); 7.0 (d, 8 Hz, H-5'); 6.5 (d, 2 Hz, H-8); 6.26 (d, 2 Hz, H-6); 5.9 (s, H-1"); 4.5 (d, 5 Hz, H-2"); 4.0 (q, 5 and 8 Hz, H-3"); 3.4 (q, 8 and 10 Hz, H-4"); 3.2 (q, 6 and 10 Hz, H-5"); 1.4 (s, CH_3); 1.34 (s, CH_3); 0.85 (d, 6 Hz, CH_3-6").

Pentaacetate of (IV) (V). mp 92-94°C, $[\alpha]_D^{20}$ −64.9° (c 2.2; acetone). Fragment of the PMR spectrum in $CDCl_3$ (ppm): 5.83 (s, H-1"); 4.76 (q, 8 and 10 Hz, H-4"); 4.58 (d, 5 Hz, H-2"); 4.2 (q, 5 and 8 Hz, H-3"); 3.2 (q, 6 and 10 Hz, H-5"); 2.44 (s, 3 H, 5-OAc); 2.30 (s, 9 H, 3',4',7-OAc); 2.04 (s, 3 H, 4"-OAc); 1.54 (s, CH_3); 1.40 (s, CH_3), 0.7 (d, 6 Hz, CH_3-6").

Quercitrin Heptaacetate (VI). mp 160-163°C (from ethanol), $[\alpha]_D^{20}$ −223° (c, 0.7; acetone). Fragment of the PMR spectrum in $CDCl_3$ (ppm): 5.68 (q, 2 and 3 Hz, H-2"); 5.6 (d, 2 Hz, H = 1"); 5.2 (q, 3 and 10 Hz, H-3"); 4.86 (t, 10 Hz, H-4"); 3.24 (q, 6 and 10 Hz, H-5"); 0.84 (d, 6 Hz, CH_3-6"); and the signals of seven acetoxy groups at 2.4 (3 H), 2.26 (6 H), 2.23 (3 H), 2.06 (3 H), and 1.9 (6 H).

CONCLUSION

1. With lower ketones (acetone, methyl ethyl ketone) and weakly acid media, flavonoid rhamnosides and rutinosides form alkylidene derivatives at the cis-diol groupings of the rhamnose residues.

2. It has been found that the formation of alkylidene derivatives of flavonoid glycosides is a side reaction that takes place during the chromatographic process on an "acid" polyamide sorbent.

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