AMORPHIGENOL β-D-GLUCOPYRANOSIDE FROM AMORPHA

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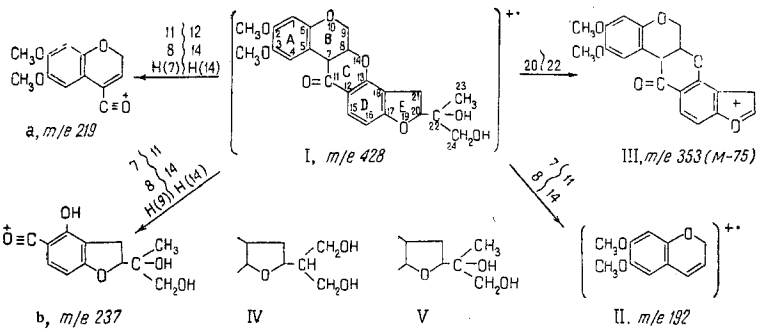
Several rotenoids, including amorphin and amorphigenin β-D-glucoside have previously been isolated from plants of Amorpha [1–3].

In a chromatographic study of extracts from the seeds of Amorpha fruticosa, A. angustifolia, A. canescens, A. frangrans, A. californica, A. glabra, A. microphylla, A. nana, A. croceolanata, and A. caroliniana we have found, in addition to those mentioned, other substances of rotenoid nature. The qualitative compositions of the rotenoids on the chromatograms of all these species of Amorpha were the same, and there were differences only in the relative amounts of particular components. For this reason, for a more detailed study of the rotenoids we used extracts of A. fruticosa as the plant richest in rotenoids [1].

The chromatography in a thin layer of silica gel of a chloroformic-methanolic extract gave three groups of five rotenoids differing in polarity: 1) the most polar, the amorphin group; 2) with medium polarity, the amorphigenin β-D-glucoside group; and 3) the least polar, the amorphigenin group [2]. In the defatting process, the last group passed partially into the petroleum ether.

The most polar group, consisting of amorphin and small amounts of another two substances, was not studied in detail. The other two groups of rotenoids were first separated from one another by coarse fractionation, and then each fraction was again separated into narrower fractions. From the low-polarity group we isolated, in addition to 7, 8-dehydroamorphigenin and amorphigenin, a new substance of rotenoid nature with the composition $C_{23}H_{24}O_8$ which we have called "amorphigenol." From the medium-polarity group we obtained another new rotenoid compound with the composition $C_{29}H_{34}O_{13}$, which proved to be a glycoside of amorphigenol. The acid hydrolysis of this compound always gave, in addition to D-glucose and amorphigenol, a certain amount of amorphigenin, $C_{23}H_{22}O_7$. A more detailed study of the hydrolysis process showed that two processes took place simultaneously: 1) hydrolysis of the glycoside with the formation of amorphigenol and D-gluocose, and 2) the conversion of the blycoside into amorphigenin β-D glucopyranoside [3] with the subsequent hydrolysis of the latter to amorphigenin and glucose. It became clear that the new glycoside is amorphigenol β-D-glucopyranoside, and its aglycone is similar instructure to amorphigenin.

The structure of amorphigenol was established mainly by studying its NMR, mass, and IR spectra. In the IR spectrum there are two narrow absorption bands at 3497 and 3407 cm^{-1}, which permitted the assumption that there are two hydroxyls in amorphigenol. The mass spectrum of amorphigenol (I) confirms its rotenoid nature [4, 5] [molecular ion (M^+ 428) heavier than that of amorphigenin by 18 units, i. e., by one molecule of water]. The existence of the maximum peak with m/e 192 (II) shows that the benzopyran part (rings A and B) of the amorphigenol molecule has the structure common to the majority of rotenoids.

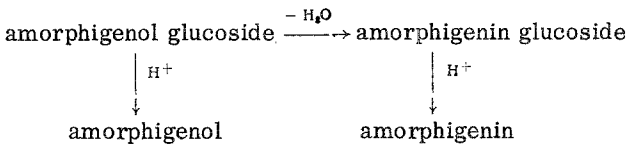


In addition, in the spectrum of amorphigenol there is a peak with m/e 353 (M − 75) which may be due to the splitting out of a side chain if the double bond present in amorphigenin and rotenone ($C_{(22)}$=$C_{(23)}$) is hydrated. This

process forms a stable oxonium ion (III). When the double bond is present, the splitting out of the side chain is unlikely and, consequently, in the spectra of amorphigenin and rotenone the analogous peak is small [4]. A confirmation of the fact that in amorphigenol, unlike amorphigenin, the double bond is hydrated is the presence in its spectrum of an ion with m/e 237 (b), together with an ion having m/e 219 (a). In the spectrum of amorphigenin, however, fragments a and b have the same mass and therefore the peak with m/e 219 possesses a double intensity [4].

The final choice of the two possible structures of the side chain of amorphigenol (IV and V) was made on the basis of the NMR spectrum, in which a new, in relation to amorphigenin, three-proton singlet appeared at τ 8.56 ppm, corresponding to a C-methyl group. These facts unambiguously determine the structure of amorphigenol as 22, 24-dihydroxy-22, 23-dihydrorotenone (I). It is not excluded that the 6', 7'-dihydroxy-6', 7'-dihydrorotenone found among the products of the enzymatic hydroxylation of rotenone (and also obtained synthetically) is a racemic form of natural amorphigenol [6].

After the structure of amorphigenol had been established, it became clear that the partial conversion of its glycoside into an amorphigenin glycoside on hydrolysis takes place as a result of the dehydration of the aglycone with the formation of a $C_{(22)}=C_{(23)}$ double bond.



Consequently, in the native compound the hydroxyl attached to the sugar moiety is unaffected on dehydration and the glycoside of amorphigenol has the structure of 22, 24-dihydroxy-22, 23-dihydrorotenone 24-O-β-D-glucopyranoside.

EXPERIMENTAL

The work was carried out with KSK silica gel, alumina for chromatography, and type "M" (Leningrad) chromatographic paper. The following systems of solvents were used: 1) butan-1-ol–acetic acid–water (4 : 1 : 5); 2) benzene–methanol in various ratios.

The rotenoids were revealed on the chromatograms with conc. H_2SO_4 containing 1 ml of a 5% solution of ferric chloride in 100 ml. The sugars were revealed with aniline phthalate.

The UV spectra were recorded in ethanol on a Hitachi instrument, the IR spectra on a UR-10 instrument in KBr, and the NMR spectra on a H-60 Hitachi instrument in deuteropyridine with hexamethyldisiloxane as internal standard. The mass spectrum was obtained on an MKh-1303 instrument at 120° C with an ionizing voltage of 40 V.

The elementary analyses of the new compounds corresponded to the calculated figures.

Extraction of Amorpha seeds. The comminuted seeds of Amorpha fruticosa, A. angustifolia, A. canescens, A. frangrans, A. californica, A. glabra, A. microphylla, A. nana, A. croceolanata, and A. caroliniana were separately defatted in the cold by steeping with petroleum ether.

The defatted seeds of each species of Amorpha were extracted separately at room temperature with a mixture of chloroform and methanol (9 : 1). The extracts were concentrated by evaporation and chromatographed on a thin layer of silica gel on long plates (19 cm) in system 2 (9 : 1). In all species of Amorpha, rotenoids with R_f 0.25–0.45 (three spots), 0.09–0.12 (three spots), and 0.03–0.05 (one strong and two weak spots) were found.

Separation of the rotenoids of A. fruticosa. The chloroformic-methanolic extract was evaporated to dryness and the residue was dissolved in hot methanol. The amorphin that crystallized out on cooling was separated off, and the solution was evaporated to 1/10 of its original volume, and again the amorphin that deposited was separated off. The mother solution was evaporated to dryness. The dry residue was separated on a column of silica gel (100 g of silica gel to 1 g of substance) in system 2 (9 : 1). The separation was monitored on a thin layer of silica gel in system 2 (3 : 1). Two groups of fractions were collected, containing rotenoids with R_f 0.25–0.45 and 0.09–0.12.

Isolation of amorphigenol (I). The group of fractions with R_f 0.25–0.45 was combined and evaporated to dryness.

The dry residue was transferred to a column of alumina (1 : 300) and separated in system 2 (10 : 1). The separation was monitored on a thin layer of silica gel in system 2 (4 : 1): the first fractions contained amorphigenin (R_f 0.45) and the subsequent ones contained 7, 8-dehydroamorphigenin (R_f 0.40). The compositions of the substances isolated coincided completely with those given in the literature [1–3] and with authentic samples.

The last fractions contained amorphigenol. The fractions were combined and evaporated, and white crystals of amorphigenol deposited in the form of needles, $C_{23}H_{24}O_8$ (elementary analysis), with mp 195–196° C; $[\alpha]_D^{20}$ −124° (c 0.47; ethanol).

The substance showed the Durham and Goodhue reactions, and with Kiliani's reagent they gave a cherry-red color. IR spectrum, cm^{-1}: 3497, 3407 (OH), 1671 (>CO). UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 239, 298 mμ (log ε 4.10, 4.21). In the NMR spectrum in the strong-field region in addition to the peaks of two O—CH_3 groups (6.38 and 6.43 ppm) there was the peak of a C—CH_3 group (τ 8.56 ppm). The mass spectrum showed peaks with m/e 428 (M^+), 413, 397, 353, 237, 219, 192.

Isolation of amorphigenol β-D-glucopyranoside. The second group of fractions (R_f 0.09–0.12) was combined and evaporated to dryness and the residue was separated on a column of silica gel (1 : 300) in system 2 (9 : 1). The separation was monitored in a thin layer of silica gel in the same system. Amorphigenin β-glucopyranoside was isolated first (R_f 0.12) [3]. Its properties were completely identical with those of an authentic sample.

Then fractions containing amorphigenol β-D-glucopyranoside (R_f 0.09) were collected. On evaporation, faintly yellowish crystals deposited with mp 189–192° C (decomp.); $[\alpha]_D^{20}$ −94.7° (c 1.3; methanol). UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 238, 297 mμ (log ε 4.06; 4.11).

The substance is soluble in methanol and chloroform and, on heating, in water. It gives the Durham and Goodhue color reactions for rotenoids, and with Kiliani's reagent forms a cherry-red coloration.

Hydrolysis of amorphigenol β-D-glucopyranoside. A mixture of 50 mg of the glycoside and 10 ml of 5% H_2SO_4 was heated in the boiling water bath. Hydrolysis was monitored in a thin layer of silica gel in system 2 (4 : 1). An hour after the beginning of hydrolysis the solution still contained the initial glycoside, and amorphigenin β-D-glucose was also detected. The precipitate was found to contain amorphigenin and amorphigenol. After two hours' heating, the glycosides had disappeared from the solution. The precipitate was filtered off, washed with water, dried, and separated in a column of alumina in system 2 (10 : 1). Amorphigenin and amorphigenol were isolated. The two compounds gave no depression of the melting point and their spectra and physicochemical properties were identical with those of the respective substances isolated directly from the extract.

The hydrolysate was neutralized with barium carbonate and chromatographed on paper in system 1 with reference materials. A spot was found at the level of D-glucose.

CONCLUSIONS

1. A third new rotenoid glycoside $C_{29}H_{34}O_{13}$, amorphigenol β-D-glucopyranoside, and its aglycone have been isolated from the seeds of Amorpha fruticosa.

2. A study of its chemical properties and its UV, IR, NMR, and mass spectra has shown that the aglycone amorphigenol has the structure of 22, 24-dihydroxy-22, 23-dihydrorotenone, and the glycoside is 22, 24-dihydroxy-22, 23-dihydrorotenone-24-β-D-glucopyranoside.

3. The presence of amorphin, amorphigenin, 7, 8-dehydroamorphigenin, amorphigenin β-D-glucopyranoside, amorphigenol, and amorphigenol β-D-glucoside in ten species of plants of the genus Amorpha has been established chromatographically.

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