STRUCTURE OF AMORPHOL – A ROTENOID BIOSIDE FROM PLANTS OF THE GENUS Amorpha

A. U. Kasymov, E. S. Kondratenko, and N. K. Abubakirov

UDC 547.918:547.972/973

We have previously reported that chloroform-methanolic extracts of the seeds of ten species of Amorpha contain three groups of rotenoids [1]. The qualitative compositions of the rotenoids were similar for all the species studied, and the plants differed only by greater or smaller amounts of the individual components. We have now made a detailed study of extracts of the fruit of A. fruticosa L., containing very large amounts of rotenoids.

The initial coarse separation of the rotenoids was performed, with respect to their solubilities, in various organic solvents. We later succeeded in developing a method of separating and isolating individual rotenoids using thin-layer and column chromatography on alumina and silica gel (Scheme 1). The group of substances of low polarity was separated satisfactorily on alumina in system 1 (see Experimental), while for the rotenoids of medium and high polarity separation on KSK silica gel in systems 2 and 3, respectively, proved to be the most successful.

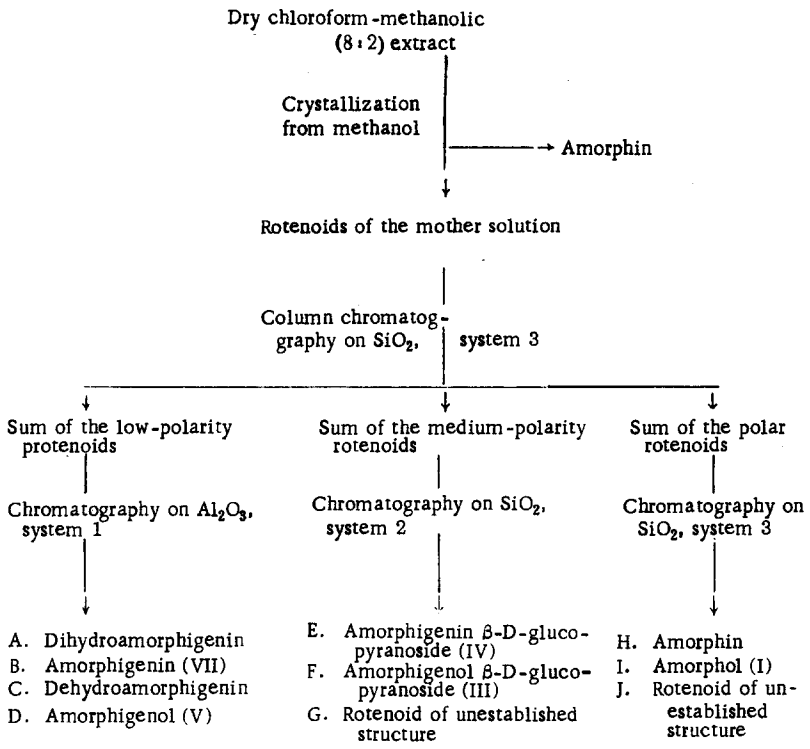
The rotenoids isolated were given the following designations: 1 – low-polarity group: substances A, B, C, and D; 2 – rotenoids of medium polarity: E, F, and G; 3 – most polar group: H, I, and J.

The rotenoids of the low-polarity group were studied fully: for substance A the structure of 24-hydroxy-22,23-dihydrorotenone has been proposed [2]; substance B has been identified as amorphigenin [3] and substance C as 7,8-dehydroamorphigenin, previously obtained synthetically from amorphigenin [4]; and substance D, given the name amorphigenol, has the structure of 22,24-dihydroxy-22,23-dihydrorotenone [1]. Of the rotenoids of the middle group, the first two components have been studied. Compound E is amorphigenin β-D-glucopyranoside [5], and F is amorphigenol β-D-glucopyranoside [1]. The main component of the total rotenoids of Amorpha is substance H – amorphin [6]. The present paper reports the results of a study of the glycoside (I) isolated from the fraction of the most polar group of rotenoids (the amount of this substance is 0.003-0.006% on the absolutely dry weight of the raw material).

Qualitative reactions [7, 8] show the rotenoid nature of compound (I). Its UV spectrum has a curve very similar to the spectra of amorphin and amorphigenin, with the two maxima at 237 and 296 nm that are characteristic for this class of plant substances.

Information on the structure of the new rotenoid (I) (Scheme 2) was obtained by its hydrolysis with acids of various concentrations and by methylation in the sugar moiety followed by the hydrolysis of the permethylate. The precipitate after the hydrolysis of glycoside (I) with 1.8% sulfuric acid was found to contain amorphigenol (V), its β-D-glucopyranoside (III), and also amorphigenin (VII) and its β-D-glucopyranoside (IV). The prolonged boiling of the glycoside in 5% sulfuric acid led to the predominant formation as a precipitate of a single product the properties of which were identical with those of amorphigenin. A similar phenomenon has been observed previously in a study of amorphigenol β-D-glucopyranoside [1]. The action of acidic agents on this glycoside (III) leads to two processes in parallel: hydrolysis of the glycoside and dehydration with the conversion of the amorphigenol into amorphigenin. Obviously, in this case as well, the aglycone of the new glycoside is amorphigenol, $C_{23}H_{24}O_8$ (V).

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 464-468, July-August, 1974. Original article submitted April 23, 1973.



Scheme 1

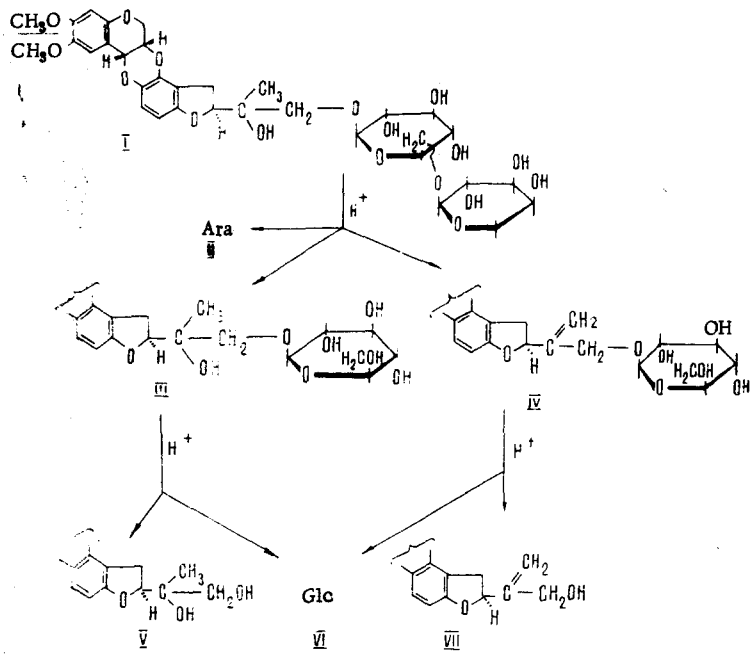
In the filtrate after neutralization, D-glucose and L-arabinose were found. The results of the gas–liquid chromatography of the silyl derivative confirm the qualitative composition of the sugars and show their ratio as 1:1. The same result is given by calculations from the yield of aglycones.

Summarizing what has been said, we may conclude that the new rotenoid which we have called amorphol (I) is a bioside of amorphigenol and has the composition $C_{34}H_{42}O_{17}$. The formation of amorphigenin and its β-D-glucopyranoside in the hydrolysis of amorphol is a consequence of the dehydration of the aglycone. The monosides of amorphigenol and amorphigenin formed in the hydrolysis of amorphol differ only in the structure of the aglycone. Consequently, the D-glucose residue in amorphol is attached directly to the aglycone by the primary hydroxy group in the pyranose form, and the glucosidic bond has the β-configuration.

The question of the size of the oxide ring and the position of attachment of the arabinose to the glucose was answered by the methylation of amorphol. Hydrolysis of a permethylate of the glycoside gave 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-arabinose. This shows that arabinose in the pyranose form is the terminal sugar and is attached to the glucose by a 1→6 bond.

The configuration of the L-arabinose–D-glucose bond was calculated from the difference in the molecular rotations of the diglycoside (I) and the monoside (III) of amorphigenol. The difference between the molecular rotations of amorphol ($[M]_D$ −570.92°, ethanol) and amorphigenol β-D-glucopyranoside ($[M]_D$ −559.29°, ethanol) is −11.63°. For methyl α-L-arabopyranoside $[M]_D$ is +28.4° (water) and for methyl β-L-arabopyranoside $[M]_D$ is +403.34° (water) [9]. These facts show the α-configuration between the L-arabinose and the D-glucose. Consequently, amorphol, like amorphin, is a glycoside of the disaccharide vicanose [10] with the structure of 24-O[α-α-L-arabopyranosyl-(1→6)-β-D-glucopyranoside] of 22,24-dihydroxy-22,23-dihydrorotenone (I).

Amorphol is the fourth representative of a new class of plant glycosides containing rotenoids as aglycones. Previously, no compounds of glycosidic nature were known among this class of plant substances. So far, rotenoid glycosides have been found only in plants of the genus Amorpha, but there is every reason to assume that they are more widespread in the vegetable kingdom. All the rotenoids studied hitherto – in particular, those from such plants as Derris, Tephrosia, and Lonchocarpus – have been isolated by extraction with nonpolar and slightly polar solvents. In such extracts the presence of hydrophilic compounds of glycosidic nature is, of course, difficult to expect. Apart from this, in the process of isolating rotenoids the plant extract is not infrequently subjected to treatment with strong acids [11]. A direct consequence



Scheme 2

of such a severe operation is the destruction of native substances. In our view, it is not excluded that compounds of glycosidic nature will be obtained in a renewed study of plants containing rotenoids with the use of modern fine methods of isolation and separation.

EXPERIMENTAL

Thin-layer chromatography (TLC) was carried out on KSK silica gel and alumina (activity grade II-III); for paper chromatography (PC) we used paper of the "slow" type and the following solvent systems: benzene–methanol – 1) (9 : 1), 2) 6 : 1, 3) 4 : 1; 4) butan-1-ol–acetic acid–water (4 : 1 : 5); 5) butan-1-ol–pyridine–water (6 : 4 : 3); 6) benzene–acetone (2 : 1); and 7) water-saturated methyl ethyl ketone.

The glycosides, genins, and methylated glycoside were revealed with concentrated sulfuric acid containing 1 ml of 5% aqueous ferric chloride in 100 ml of acid, and the sugars were revealed with o-toluidine salicylate. The gas–liquid chromatography of the silylated methyl glycosides was performed by T. T. Gorovits on a Tsvet-2 chromatograph [12, 13] using a steel column (2 m × 3 mm containing 5% of G-30M silicone phase on Celite 545 (80–100 mesh), column temperature 160°C; carrier gas – hydrogen, at a rate of 55 ml/min.

Isolation of Amorphol (I). The air-dry comminuted fruit of Amorpha (5 kg) was defatted with petroleum ether. In the extract, TLC showed the presence of the low-polarity group of rotenoids (R_f 0.67-0.57 in system 3). Then the plant material was extracted with diethyl ether. In this extract TLC showed the presence of, in addition to the low-polarity group, rotenoids of medium polarity (R_f 0.47-0.30 in system 3). Finally, the raw material was extracted with chloroform–methanol (8 : 2). This last extract contained, in addition to the two groups mentioned, the most polar rotenoids (TLC, R_f 0.16-0.10 in system 3). Concentration to half its volume of the chloroform-methanolic extract led to the precipitation of crude amorphin. The mother solution was evaporated to dryness, and the residue was heated with methanol. The undissolved matter (additional amount of amorphin) was separated off, and the mother solution was evaporated to dryness (see Scheme 1). The yield of crude amorphin was 45 g, and the yield of extractive substances was 105 g.

In 10-g portions, the dry residue was mixed with twice its weight of silica gel and transferred to columns each containing 1.5 kg of silica gel. Elution was performed successively with systems 1, 2, and 3, 50-ml fractions being collected. The process of separation was monitored by TLC on silica gel in the same systems. The eluates containing rotenoids H and I were combined, evaporated to dryness, and rechromatographed on a column with an increase in the ratio of silica gel to the mixture of rotenoids to 1 : 300. Concentration of the eluates containing solely the glycoside (I) gave about 15-17 mg of a yellowish

powder of amorphol with mp 159-162°C (decomp.), $[\alpha]_D^{22}$ −96.6° $_0$c 2.07; methanol), −79.0° (c 0.47; ethanol). The Durham [7] and Goodhue [8] reactions are positive, and with the Keller–Kiliani reagent amorphol gives a cherry-red coloration [6]. UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 237 and 296 nm; log ε 4.09 and 4.22.

Hydrolysis of Amorphol (I) with 1.8% Sulfuric Acid. A mixture of 40 mg of amorphol and 20 ml of 1.8% sulfuric acid was heated for 3 h. After the mixture had cooled, the precipitate was filtered off and washed with water to neutrality, and in it amorphigenin (VII), amorphigenol (V), amorphigenin β-D-glucopyranoside (IV), and amorphigenol β-D-glucopyranoside (III) were detected by TLC. The precipitate (18 mg) was separated on a column of alumina (20 g) in system 1, giving 5 mg of amorphigenin and 4 mg of amorphigenol, these being identified by their melting points and mixed melting points with authentic samples.

In the filtrate after neutralization with barium carbonate, D-glucose (VI) and L-arabinose (II) were identified by paper chromatography in systems 4 and 5.

Hydrolysis of Amorphol (I) with 5% Sulfuric Acid. With heating, 40 mg of amorphol was dissolved in 20 ml of 5% sulfuric acid. The solution was boiled for 4 h with constant stirring. The precipitate was filtered off (23 mg), and after recrystallization from methanol it had mp 191-193°C, $[\alpha]_D^{20}$ −135° (c 0.1; ethanol). The crystals isolated were identified by a mixed melting point and by the TLC method on silica gel in system 1 with an authentic sample of amorphigenin (VII). In the hydrolyzate, after neutralization with barium carbonate, D-glucose and L-arabinose were identified by PC in systems 4 and 5 with markers.

Methylation of Amorphol (I). Hakomori's method was used to methylate 50 mg of amorphol [10]. The permethylate was heated at the boil in a 5% aqueous methanolic solution of sulfuric acid for 1 h. Then the mixture was diluted with water, and it was heated for another h. The hydrolyzate was neutralized with barium carbonate, and 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-arabinose were identified by TLC in system 6 and PC in system 7 with markers.

SUMMARY

A new glycoside with a rotenoid aglycone – amorphol – has been isolated from the fruit of Amorpha fruticosa L. It has been established that amorphol is a bioside of amorphigenin and has the structure of the 24-O-[O-α-L-arabopyranosyl-(1→6)-β-D-glucopyranoside] of 22,24-dihydroxy-22,23-dihydrorotenone.

LITERATURE CITED

1. A. U. Kasymov, E. S. Kondratenko, Ya. V. Rashkes, and N. K. Abubakirov, Khim. Prirodn. Soedin., 197 (1970).
2. A. U. Kasymov, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prirodn. Soedin., 115 (1972).
3. A. U. Kasymov, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prirodn. Soedin., 307 (1967).
4. E. S. Kondratenko and N. K. Abubakirov, Uzb. Khim. Zh., No. 5, 66 (1961).
5. A. U. Kasymov, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prirodn. Soedin., 326 (1968).
6. E. S. Kondratenko and N. K. Abubakirov, Dokl. Akad. Nauk UzSSR, No. 10, 35 (1960).
7. H. Jones and G. Smith, Ind. Eng. Chem., Anal. Ed., 5, 75 (1933); Chem. Zentr., II, 582 (1933).
8. L. O. Goodhue, J. Assoc. Offic. Agric. Chemists, 19, 118 (1936); Chem. Zentr., II, 2602 (1936).
9. Beilsteins Handbuch der Organischene Chemie, Vol. 31, Berlin (1938), p. 45.
10. Dictionary of Organic Compounds, Vol. 5, Eyre and Spottiswoode, London (1965), p. 3244.
11. P. E. Clark, J. Amer. Chem. Soc., 53, 313 (1931).
12. C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, J. Amer. Chem. Soc., 85, 2497 (1963).
13. T. T. Gorovits, Khim. Prirodn. Soedin., 49 (1969).
14. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).