CONSTITUTION OF MAXIMA SUBSTANCE A

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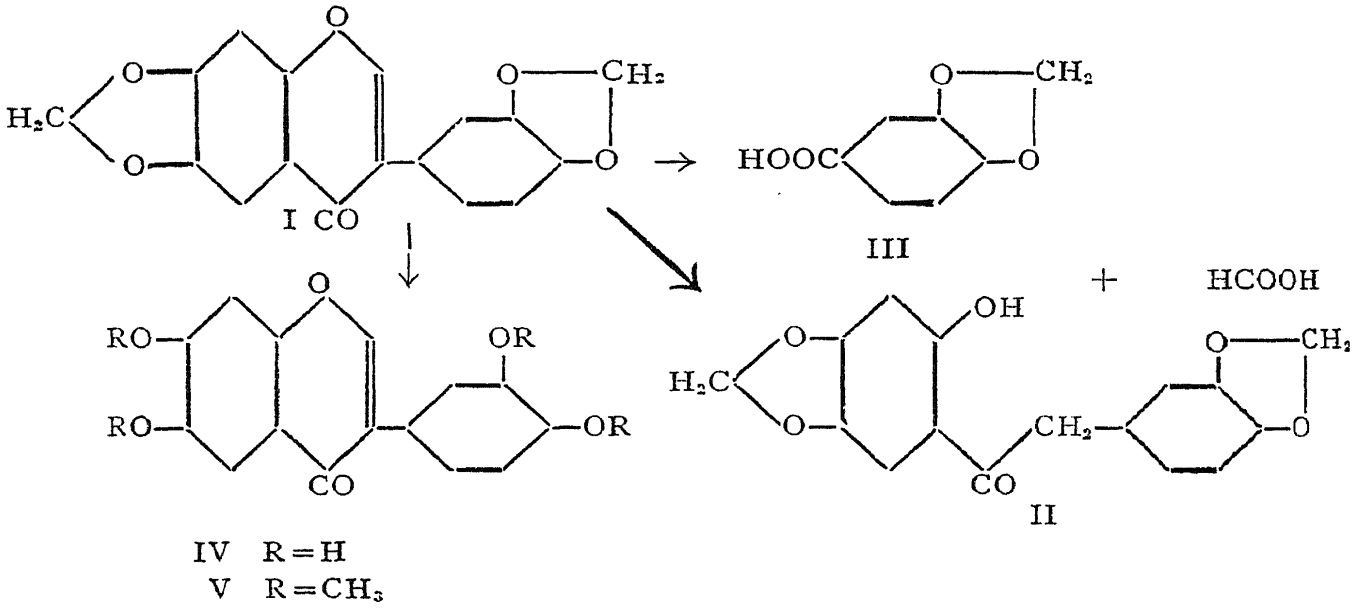
Received September 4, 1956

Maxima substance A was isolated from the chloroform extract of the roots of *Tephrosia maxima* Aers.[1] It forms colourless plates from benzene melting at 227–29° and has the probable molecular formula $C_{17}H_{10}O_6$. It did not answer the rotenoid colour reactions and the substance was recovered after treatment with iodine and sodium acetate in boiling alcohol. It was insoluble in aqueous sodium hydroxide and did not give any colour with ferric chloride. It was recovered even after boiling for three hours with acetic anhydride and sodium acetate. It did not form a derivative with 2:4-dinitrophenylhydrazine. It dissolved in concentrated sulphuric acid forming a yellow solution but the substance was recovered on dilution with water. It contained no methoxyls (micro-Zeisel) but gave positive test for the methylenedioxy grouping (green colour with gallic acid and sulphuric acid).

Boiling the substance with 12% alkali gave rise to formic acid and a phenolic ketone (II) melting at 142–44° and having a molecular formula $C_{16}H_{12}O_6$. Formic acid was identified by its reducing action on mercuric chloride and by its reduction to formaldehyde which was again identified by its colour reactions. On oxidation of maxima substance A with alkaline hydrogen peroxide, piperonylic acid (III) was obtained. These reactions suggest for the substance an isoflavone structure with a methylenedioxy group at 3′:4′ position. An isoflavone with a methylenedioxy group at 3′:4′ position should have the molecular formula $C_{16}H_{10}O_4$. Hence Maxima substance A, which has the formula $C_{17}H_{10}O_6$ will be an isoflavone with two methylenedioxy groups. This was confirmed by the fact that treatment of maxima substance A with anhydrous aluminium chloride in boiling benzene solution gave a tetrahydroxy-isoflavone (IV) due to demethylenation. This demethylenation did not go well when hydriodic acid or hydrobromic acid was employed. The tetrahydroxy compound (IV) was characterised by methylating it with dimethyl sulphate and potassium carbonate in acetone solution when it yielded the tetramethyl ether (V). Since there is only one methylenedioxy group in the side phenyl nucleus, the second methylenedioxy group should be located in the benzene ring of the benzo-pyrone part of the molecule. There are three possibilities for the position of this second methylenedioxy group, namely, 5:6, 6:7 or 7:8. An isoflavone with a hydroxyl in position 5 should be sparingly soluble in aqueous alkali and should give a positive

Wilson's boric-citric reaction.[2] Since the tetrahydroxyisoflavone (IV) was easily soluble in aqueous alkali and gave a negative Wilson's boric-citric reaction, the 5:6 position for the second methylenedioxy group in the parent compound seems to be ruled out. The phenolic ketone (II) failed to give a blue colour with 2:6-dichloroquinonechlorimide reagent under the conditions described by Hochberg *et al.*[3] This may mean that in this compound the position *para* to the phenolic hydroxyl is not free. These observations seem to indicate that the second methylenedioxy group in Maxima substance A is probably in the 6:7 position. The 7:8 position for the methylenedioxy grouping does not seem very likely in view of the fact that few 8-hydroxy- or 8-methoxyisoflavones have so far been reported to occur in nature. A rigid proof for the 6:7 position for the methylenedioxy group is very desirable and possibilities in this direction are being explored.



EXPERIMENTAL

Maxima substance A was obtained as colourless crystalline plates, m.p. 227–29° from benzene (Found: C, 66·1, H, 3·7%; OCH_3, Nil. $C_{17}H_{10}O_6$ requires C, 65·8; H, 3·3%).

Hydrolysis of maxima substance A

(*a*) *With alkaline hydrogen peroxide.*—The substance (250 mg.) was dissolved in 25 c.c. of 5% alcoholic potassium hydroxide (made with 80% alcohol) by warming for 15 minutes and 30% hydrogen peroxide was added little by little (total 3 c.c.). When the effervescence ceased, the solution was warmed on a water-bath to decompose the excess of hydrogen peroxide, diluted to 100 c.c., acidified with dilute hydrochloric acid and extracted with ether. The ether extract was divided into acidic, phenolic and neutral fractions by extracting with 5% aqueous sodium bicarbonate and 5% aqueous

sodium hydroxide. The acidic fraction (100 mg.) on two crystallisations from acetone-petroleum ether gave colourless plates melting at 225–28°. It did not give any colour with ferric chloride but gave a green colour with gallic acid and sulphuric acid [Found: C, 58·3; H, 4·1%. $C_8H_6O_4$ (piperonylic acid) requires C, 57·8; H, 3·6%]. The mixed m.p. with an authentic specimen of piperonylic acid melting at 230–32° was 228–30°.

The phenolic fraction (80 mg.) on crystallisation from benzene-petroleum ether gave colourless shining needles, initially melting at 131–32°, again crystallising and remelting at 142–44°. When the substance was dried at 80° for three hours it melted directly at 142–44°. It gave a reddish brown colour with ferric chloride. It formed a greenish yellow solution in concentrated sulphuric acid which turned deep green when warmed with a speck of gallic acid [Found: C, 64·6; H, 4·5%. $C_{16}H_{12}O_6$ (phenolic ketone II) requires C, 64·0; H, 4·0%].

The 2:4-dinitrophenylhydrazone of the phenolic ketone (II) prepared in the usual manner crystallised from chloroform-methanol as bright yellow plates, m.p. 239–41° (Found: C, 55·7; H, 3·7; N, 12·1%. $C_{22}H_{16}O_9N_4$ requires C, 55·0; H, 3·4; N, 11·7%).

(*b*) *With* 12% *sodium hydroxide.*—Maxima substance A (436 mg.) was suspended in a solution of 12% sodium hydroxide made with 50% alcohol (50 c.c.) and refluxed until the whole of the substance went into solution (about 30 minutes), cooled, diluted with 100 c.c. of water, made acidic to congo red with 20% phosphoric acid and cooled in the ice-chest for one hour. The solid (X) that separated was filtered and washed free from acid with water. The filtrate and washings were extracted with petroleum ether (3 × 100 c.c.). The petroleum ether was washed with small quantities of water and these washings and aqueous layer (Y) were reserved for the estimation of formic acid.

The residue from the petroleum ether extract and the solid (X) were mixed, dissolved in ether and washed with 5% aqueous sodium bicarbonate. The ether was evaporated and the residue (300 mg.) was crystallised from methanol. Colourless plates melting at 131–32°, solidifying and remelting at 142–44° were obtained. This substance was identical with the phenolic ketone obtained in experiment (*a*).

Formic acid.—The aqueous liquid (Y) was steam distilled and about 500 c.c. of distillate was collected. The formic acid in the distillate was determined by titration with N/20 sodium hydroxide using phenolphthalein as indicator (yield: 50% of theoretical).

The neutralised distillate was reduced to a small bulk. A portion of it was reduced with magnesium and sulphuric acid (1 : 4) cooling the solution in ice. The resulting solution answered all the qualitative tests for formaldehyde. Another aliquot portion of the neutralised distillate was used for the estimation of formic acid by Riesser's method[4] (reduction of mercuric chloride to mercurous chloride and back oxidation of mercurous chloride to mercuric chloride with standard iodine solution) (yield: 50% of theoretical).

(*c*) *With* 30% *potassium hydroxide*.—Maxima substance A (250 mg.) was boiled with 20 c.c. of 30% alcoholic potash for 3 hours, cooled, diluted with water (100 c.c.), acidified with dilute hydrochloric acid and extracted with ether. The ether extract was divided into acidic, phenolic and neutral fractions as usual. The only crystalline product obtained was the phenolic ketone (II).

Demethylenation of maxima substance A to 6 : 7 : 3′ : 4′-*tetrahydroxyisoflavone*.—Maxima substance A (200 mg.) in benzene (20 c.c.) was treated with anhydrous aluminium chloride (2 g.) and the mixture was boiled under reflux for 2½ hours. The mixture was cooled, the benzene solution was decanted off into another flask and the solvent was removed under vacuum. To the residues in the two flasks 1 : 1 hydrochloric acid was added to decompose the complex and the solution was extracted with ether. The yellow ether solution was extracted with 2% aqueous sodium hydroxide. The alkali-soluble portion was recovered by acidification and extraction with ether. The ether solution was dried and evaporated under vacuum. The residue was taken in the minimum amount of ethyl acetate and benzene was added drop by drop until turbidity developed. On cooling in the ice-chest resins sticking to the sides separated. The mother liquor was decanted into another vessel and the process of adding benzene and removal of resins was repeated thrice. Finally the clear benzene-ethyl acetate solution was concentrated and kept in the ice-chest. The yellow crystalline solid that separated was crystallised again from methanol-benzene. Dark yellow prisms and needles, darkening at about 270° and melting at 320° (decomp.), were obtained. The solution gave a dark green colour with alcoholic ferric chloride. No green colour was obtained on warming with concentrated sulphuric acid and gallic acid. No yellow colour was developed in Wilson's boric-citric reaction. The substance was insoluble in aqueous sodium bicarbonate and completely soluble in aqueous sodium hydroxide forming a yellow solution [Found: C, 62·5; H, 3·5%, $C_{15}H_{10}O_6$ requires C, 63·0; H, 3·5%].

6 : 7 : 3′ : 4′-*tetramethoxyisoflavone*.—The above tetrahydroxyisoflavone (40 mg.) in acetone (10 c.c.), dimethyl sulphate (0·4 c.c.) and anhydrous potassium carbonate (1 g.), were refluxed together on a water-bath for 5 hours. The acetone was decanted off, the potassium carbonate was washed with acetone, the acetone solutions were evaporated under vacuum and the residue was treated with water. The precipitated solid was filtered, washed with 2% aqueous sodium hydroxide and finally free from alkali with water. The substance was dried and purified by two crystallisations from methanol-petroleum ether. Colourless prisms (30 mg.) melting at 168–70° were obtained. The substance did not give any colour with ferric chloride [Found: C, 66·2; H, 5·7; OCH_3, 35·1%. $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3; OCH_3 (4), 36·3%].

Summary

Maxima substance A has been shown to be a dimethylenedioxy-isoflavone. One of the methylenedioxy groups is in the 3′ : 4′-position of the side phenyl nucleus; the other is most probably in the 6 : 7 position in the benzopyrone part.

Acknowledgment

We thank Mr. E. Venkata Rao for the microanalyses.

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