CHEMICAL INVESTIGATION OF INDIAN FRUITS

Part V. Constitution of Auranetin

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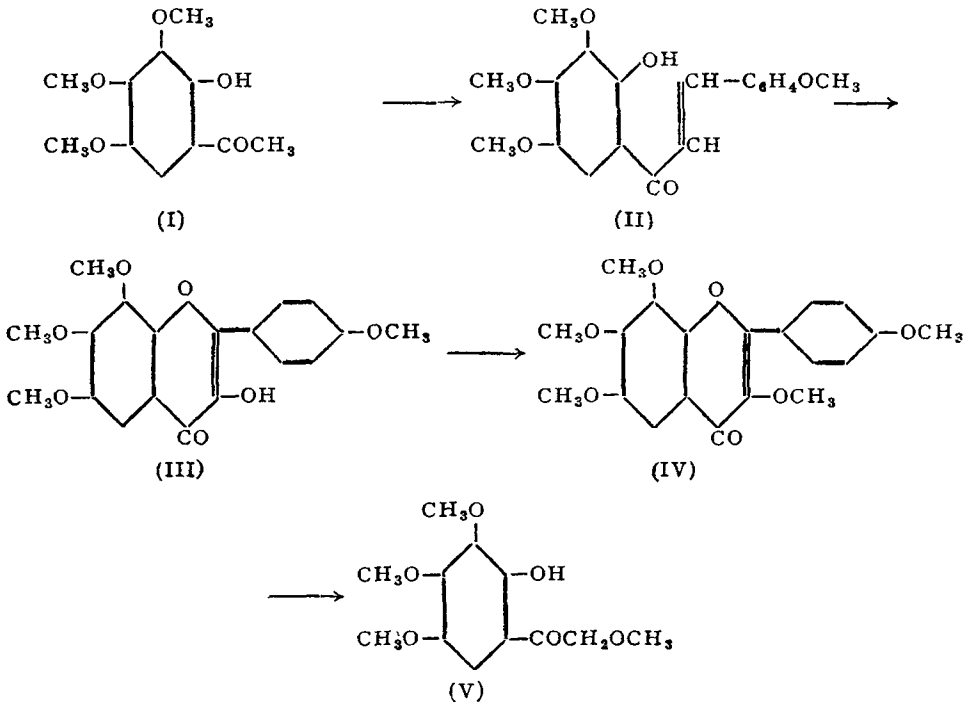
In an earlier paper (Part III[1]) was reported the isolation of a new compound in a small yield from the loose peels of kamala oranges, a local variety of *Citrus aurantium*. It was a fully methylated flavone like tangeretin and nobiletin and appeared to be a new entity found in the Citrus. It was named 'aurantin' based on its natural occurrence but it was realised later that this name had already been given to another substance isolated from the fungus *Oospora aurantia* (Cooke).[2] The name of the citrus product is now therefore changed to 'auranetin'.

The progress of work on the constitution of auranetin has been rather slow since, besides the difficulties of war years, the yield of the compound was very low and its purification was difficult. We examined similar fruits from other localities with the object of discovering a better source. So far this has been unsuccessful. The fruits from other places have not yielded any auranetin. Consequently a good portion of the earlier work had to depend on comparison with other known compounds.

The original sample of auranetin was not pure though it was crystalline. As the result of further work it has been possible to get colourless samples with a considerably higher melting point (139–40°). Its molecular formula is now definitely established as $C_{20}H_{20}O_7$ and it contains five methoxyl groups. Fission with alcoholic potash yielded anisic acid and a pale yellow ketone melting at 116–17° and having the formula $C_{12}H_{16}O_6$ with four methoxyl groups. It could not be studied further owing to lack of material. However the isolation of anisic acid narrowed down the possibilities. Auranetin was definitely different from tangeretin and pentamethyl herbacetin and the mixed melting points were lowered. The lower member of the nobiletin series (5 : 6 : 7 : 8 : 4′-pentamethoxy flavone) was then prepared.[3] It was found to melt at 150-51°. It was therefore clear that auranetin was a flavonol ether and the fairly smooth fission of the compound with alkali supported this opinion. Of the four possible structures for the flavonol pentamethyl ether, two, tangeretin and pentamethyl-herbacetin, were already

ruled out. The other two would have the 5:6:8 and 6:7:8 arrangement of methoxyl groups. The former though uncommon had recently been found in gardenin and 3:5:6:8:4′-pentamethoxy flavone[4] was therefore prepared for comparison. Its melting point was 142–43° which was very nearly that of auranetin; but a mixture of the two melted markedly lower. Thus by elimination auranetin should have the constitution of 3:6:7:8:4′-pentamethoxy flavone (IV). Flavonols with the 6:7:8-arrangement of hydroxyl or methoxyl groups have not so far been found to occur in nature. But the compound having the above constitution has been prepared a few years back by Bargellini and Oliverio[5] synthetically using the following steps.



The recorded properties of the pentamethyl ether, penta-hydroxy flavone and its acetate agreed with those of auranetin, norauranetin and its acetate. The melting points are compared in Table I. For purposes of direct comparison the compounds were synthesised. Since the original paper of Bargellini and Oliverio was not available at that time and only the abstract could be obtained, the methods of our preparation, being somewhat different, are briefly given. The agreement between the natural and synthetic samples is complete. Further the alkali fission of the synthetic pentamethyl ether yields besides anisic acid, 2-hydroxy-ω:3:4:5-tetramethoxy-acetophenone

(V) which is found to be identical with the ketone obtained from auranetin. Hence the identity of the two series, natural and synthetic, is established.

TABLE I

	Natural compounds	Synthetic compounds	
		Bargellini and Oliverio	Ours
1. Pentahydroxy compound ..	Above 360°	Above 360°	Above 360°
2. Methyl ether (Auranetin) ..	139-40°	141°	139-40°
3. Acetate ..	220-21°	216°	220-21°
4. Fission ketone ..	116-17°	..	116-17°

Thus in auranetin the citrus fruits have made another notable contribution to the chemistry of flavones. The American tangerine oranges provided the first fully methylated flavone, tangeretin[6] indicating the existence of a powerful methylation mechanism capable of attacking even the resistant 5-hydroxyl group. The Chinese mandarin oranges yielded nobiletin[7] which is not only a fully methylated flavone with six methoxyl groups, but has further a fully oxidised condensed benzene nucleus (5:6:7:8). The Indian kamala oranges have made the third contribution. Auranetin is another fully methylated compound and represents the so far unrepresented 6:7:8-combination of methoxyl groups. With this discovery the occurrence in nature of all the possible arrangements of three hydroxyl or methoxyl groups in the condensed benzene nucleus has been established. The most common are the 5:6:7 and 5:7:8 orientations and they are represented by a number of compounds. The 5:6:8-arrangement in which the 7-position is free is rare and it is represented only by the recently discovered gardenin. Similarly the 6:7:8-orientation of groups in which the 5-position is unsubstituted is equally rare and auranetin becomes the only known representative. Its evolution from the fundamental 5:7-hydroxy (quercetin) type should involve stages of oxidation of the positions 6 and 8 and reduction of the position 5 somewhat analogous to what has been suggested for gardenin.[8] In view of the considerations discussed in a recent publication[9] the following alternative seems to be the most probable.

$$5:7 \rightarrow 5:6:7 \rightarrow 5:6:7:8 \rightarrow 6:7:8$$

Though the removal of the 5-hydroxyl group may be possible at an earlier stage, oxidation of position 8 is best promoted by the presence of this group and it is therefore retained till the last stage in the above scheme,

EXPERIMENTAL

Isolation of Auranetin

The peels of the kamala oranges were sun dried and cut into small pieces and extracted with ligroin by heating on a water-bath for 10 hours; the yellow extract was poured out and the extraction repeated twice more. The extracts were combined and the solvent was distilled off under reduced pressure as competely as possible. The deep yellow oily residue was poured into an evaporating basin and the last traces of the solvent allowed to evaporate. The semi-solid product was triturated with cold methyl alcohol when most of the waxes and carotenoids remained undissolved and were filtered off. The methyl alcoholic solution was then evaporated and the remaining oily residue was dissolved in a small quantity of ether (1 volume) and diluted with four volumes of petroleum ether. A pale yellow crystalline solid began to separate in about a day; after a few days it was filtered and washed with a small quantity of petroleum ether to remove the adhering oil, and recrystallised from ether-petroleum ether mixture, when auranetin separated as almost colourless (very pale yellow) fine needles, m.p. 139–40°. (Found: in air-dried sample: C, 61·7; H, 6·0; loss on drying at 110° *in vacuo*, 4·2%; OCH_3, 40·2%; $C_{20}H_{20}O_7$, H_2O requires C, 61·5; H, 5·6; H_2O loss, 4·6%; OCH_3 for five OCH_3 groups, 39·7%. Found in the vacuum dried sample: C, 64·2; H, 5·8; OCH_3, 41·2; $C_{20}H_{20}O_7$ requires C, 64·5; H, 5·4; OCH_3, 41·7%). The other properties and the reactions of auranetin were the same as described in detail in a previous paper.[1]

Nor-auranetin

Auranetin was demethylated with hydriodic acid as already described and nor-auranetin was recrystallised from a mixture of alcohol and acetic acid. It appeared as yellow rectangular rods under the microscope and on heating turned brown at about 300°, but did not melt below 360°. (Found in air dried sample: C, 56·5; H, 3·4; and loss on drying *in vacuo* at 120°, 5·1; $C_{15}H_{10}O_7$, H_2O requires C, 56·2; H, 3·7; loss on drying 5·6%. Found in the dried sample C, 59·3; H, 3·2; $C_{15}H_{10}O_7$ requires C, 59·6 and H, 3·3%).

The colour reactions of nor-auranetin in alkaline buffer solutions were already described. In 5% sodium carbonate solution, the compound dissolved with a deep red colour which was stable for 12 hours. A small quantity of a blue precipitate separated in half an hour.

Nor-auranetin was acetylated by boiling with acetic anhydride and sodium acetate for 2 hours. The product crystallised from ethyl acetate-petroleum ether mixture as colourless needles melting at 220-21°. (Found; C, 58·3; H, 4·3; $C_{25}H_{20}O_{12}$ requires C, 58·6 and H, 3·9%.)

Fission of Auranetin

Auranetin (1 g.) was dissolved in absolute alcoholic potash (2 g. in 25 c.c.) and the solution refluxed for 6 hours on a water-bath. At the end of the period, the alcohol was removed under reduced pressure, water added and the alkaline solution acidified. The mixture was extracted with ether and the ether extract washed successively with 5% sodium bicarbonate and 5% sodium hydroxide. Each of the solutions was acidified, extracted with ether, the extract dried over anhydrous sodium sulphate and the solvent evaporated. From the bicarbonate solution was thus obtained anisic acid, its melting point being undepressed on admixture with an authentic specimen. The sodium hydroxide extract gave a pale yellow compound (ketone) which after recrystallisation from alcohol formed pale yellow rectangular prisms and melted at 116–17°. It gave a brown colour with alcoholic ferric chloride. (Found: OCH_3, 45·6; $C_{12}H_{16}O_6$, H_2O requires OCH_3, 45·2%.)

2-*Hydroxy*-3 : 4 : 5 : 4′-*tetramethoxy-chalkone*[5] (*II*)

2-Hydroxy-3 : 4 : 5-trimethoxy acetophenone[10] (I) (1 g.) and anisaldehyde (5 c.c.) were dissolved in alcohol (20 c.c.) and to the solution was added aqueous sodium hydroxide (10 g. in 10 c.c. of water) in small quantities while cooling in ice. The flask was then corked air-tight and left for 2 days with occasional shaking. The deep-red solution slowly solidified; water was then added and the solution extracted with ether to remove the unreacted aldehyde. It was acidified and again extracted with ether. The ether extract was washed with sodium bicarbonate solution to remove anisic acid, dried over anhydrous sodium sulphate and the solvent removed. A yellow oil was obtained which turned into a bright yellow crystalline solid on leaving in the refrigerator. It crystallised from alcohol as deep yellow rectangular plates, melting at 111–12°. (Bargellini and Oliverio give the melting point as 110°.)

3-*Hydroxy*-6 : 7 : 8 : 4 -*tetramethoxy flavone*[5] (III)

The above chalkone (1 g.) was dissolved in rectified spirit (25 c.c.) and aqueous sodium hydroxide (2N, 50 c.c.). To the red solution was added hydrogen peroxide (100 vols. 10 c.c.) in small quantities with cooling under the tap and vigorous shaking. A yellow solid separated during the preliminary stages which later went into the solution with a deep yellow colour. The excess of hydrogen peroxide was decomposed by heating on a water-bath and then the alcohol evaporated off. On cooling the remaining solution and acidifying it, the flavonol separated as a brown solid. Recrystallisation from rectified spirit yielded it as yellow rods which melted at 186-7°. Yield 0·4 g. (Bargellini and Oliverio record the m.p. as 187°.)

3:6:7:8:4′-*Pentamethoxy flavone*[5] (*IV*).

The 3-hydroxy compound (III) was methylated with dimethyl sulphate and freshly ignited potassium carbonate in dry acetone solution by refluxing on a water-bath for 10 hours. The acetone was distilled off, water added to the residue and the aqueous solution extracted with ether. On evaporating the ether solution a colourless solid remained which crystallised from alcohol as very pale yellow needles melting at 139–40°. Mixed melting point with the natural sample of auranetin was undepressed. (Bargellin and Oliverio[5] give the melting point of the pentamethoxy flavone as 141°.)

3:6:7:8:4′-*Pentahydroxy flavone*.[5]

The pentamethoxy flavone (0·5 g.) was dissolved in acetic anhydride (5 c.c.) and to the solution was added hydriodic acid (5 c.c. of 1·7 d.) with cooling. The mixture was gently boiled for half an hour, cooled and diluted with water saturated with sulphur dioxide. The precipitated deep yellow solid was filtered and thoroughly washed with aqueous sulphur dioxide. It was dried and recrystallised from acetic acid when it was obtained as bright yellow rectangular plates. The compound turned brown at about 300°, but did not melt below 360°. (Bargellini and Oliverio also recorded the same property).

It was identical with nor-auranetin in all its properties and colour reactions, including those given by alkaline buffer solutions. Its acetate crystallised from absolute alcohol as colourless needles melting at 220–21°. It did not depress the melting point of the acetate of nor-auranetin. (Bargellini and Oliverio give the melting point of the acetate as 216°.)

The above pentamethoxy flavone was subjected to alkaline hydrolysis as in the case of auranetin. The products were anisic acid and the 2-hydroxy-ω:3:4:5-tetramethoxy acetophenone (V) melting at 116–17°; the melting point was not depressed by admixture with the ketone obtained from auranetin. (Found: C, 52·4; H, 6·8; Loss on drying *in vacuo* at 100°, 6·9; $C_{12}H_{16}O_6$, H_2O requires C, 52·6; and H, 6·6; H_2O loss 6·6%.)

Summary

Auranetin has been obtained pure. It is a colourless solid melting at 139–40° and is a pentamethoxy flavone. By alkali fission it yields anisic acid and a ketone containing four methoxyl groups. Direct comparison with compounds belonging to the types already known to occur in nature showed that it is quite novel and should belong to 6:7:8-methoxy type. This was confirmed by the preparation of 3:6:7:8-4′-pentamethoxy flavone and its derivatives and direct comparison. The ketone obtained

by the fission of the synthetic flavone is also identical with the one derived from auranetin. The contribution of citrus fruits to flavone chemistry and the biogenesis of the auranetin group are discussed.

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