A NEW EFFECT OF HYDROGEN BOND FORMATION (CHELATION)

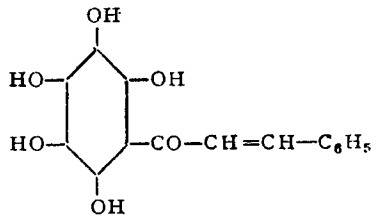
Part II. Constitution of Despedicellin, Dihydropedicinin and Isopedicin

BY G. S. KRISHNA RAO, K. VISWESWARA RAO AND T. R. SESHADRI

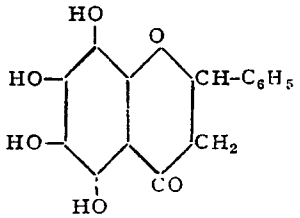
(From the Department of Chemistry, Andhra University, Waltair)

Received September 7, 1948

DESPEDICELLIN was obtained by the action of hydriodic acid on pedicellin, pedicin and pedicinin.[1] It was given the pentahydroxy chalkone structure (I). A substance of this constitution would normally be expected to have a bright red colour but despedicellin is only a pale straw yellow crystalline substance. This property would suggest that it is a flavanone (II) which should also be expected in accordance with the observations recorded in Part I of this series[2] and the explanations offered. Owing to the formation of hydrogen bonds, whenever there are two hydroxyl groups in the 2 : 6-positions of a chalkone it should be expected to undergo ready conversion into the flavanone which has a high degree of stability.



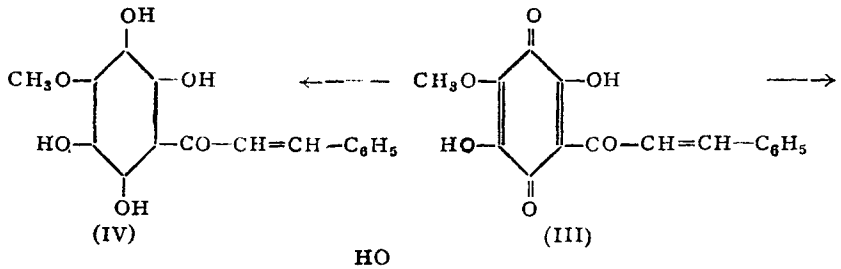
(I)



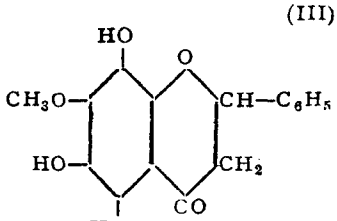
(II)

The position seems to be quite similar in the case of dihydropedicinin obtained by the reduction of pedicinin using stannous chloride and hydrochloric acid.[3] Since the constitution of pedicinin has been conclusively shown to be that of a hydroxy-quino-chalkone (III),[4] it follows that the reduction product should be either a tetrahydroxy chalkone (IV) or the corresponding flavanone (V). The lack of prominent colour even in this case would indicate that dihydropedicinin is also a flavanone (V).

In the course of the work presented in this paper a detailed study of the properties of these compounds has been made in order to get more precise information regarding their constitution. Methylation in stages using dimethyl sulphate and potassium carbonate as was described in Part I[2] in connection with naringin and naringenin was first attempted. Using five molecular proportions or excess of dimethyl sulphate despedicellin could now be readily and completely methylated to pedicellin though reports of

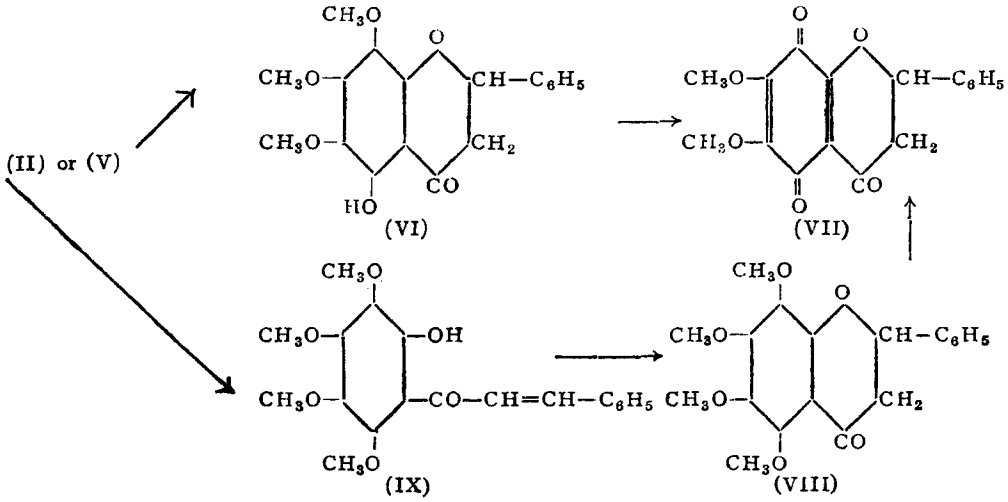


(IV) (III)



(V)

unsuccessful attempts were made by earlier authors.[1] But partial methylation with three molecular proportions of this reagent to yield the 5-hydroxy flavanone derivative has not been quite successful owing to its low melting point and high solubility in organic solvents. Hence the use of diazomethane has been examined next. It is well established that this reagent does not attack the 5-hyndroxyl group of flavanones even when used in excess. By its action on despedicellin or dihydropedicinin is obtained the same compound melting at 99–100° and having only a very pale yellow colour. It has three methoxyl groups in the molecule and gives a prominent green colour with ferric chloride. All these properties agree with the structure of 6:7:8-trimethoxy-5-hydroxy flavanone (VI). If the above compounds were chalkones, a tetramethyl ether would have resulted.



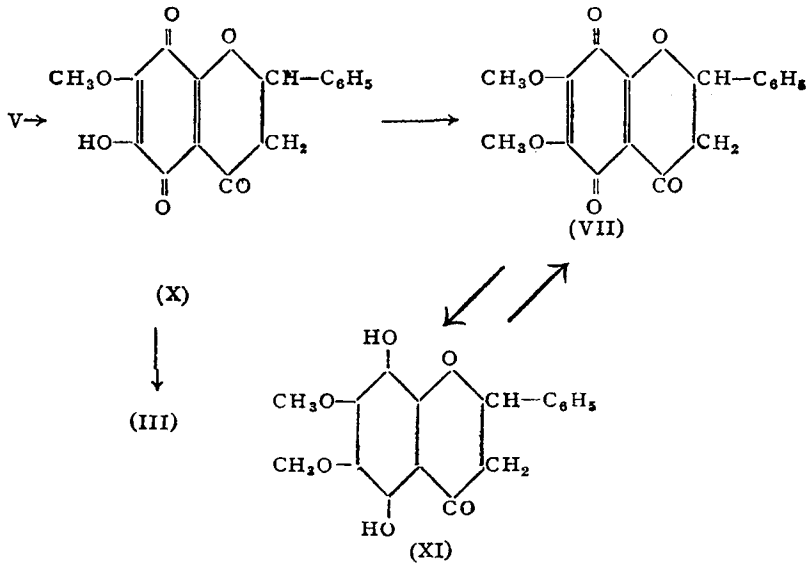
(II) or (V) (VI) (VII) (IX) (VIII)

Further the trimethyl ether (VI) undergoes oxidation with nitric acid to form a quinone (VII) which is identical with the substance obtained by the oxidative demethylation of 5:6:7:8-tetramethoxy flavanone (VIII). This last mentioned compound (VIII) is prepared by the methylation of despedicellin (II) using four molecular proportions of dimethyl sulphate and cyclising the resulting tetramethoxy chalkone (IX) by means of alcoholic hydrochloric acid.

The formation of the chalkone derivative (IX) from despedicellin (II) is in line with similar observations made in the case of naringin and naringenin,[2] where also the flavanone ring was found to open out under the conditions of this methylation as soon as the 5-hydroxyl group gets methylated. The tetramethoxy chalkone (IX) is an orange coloured liquid giving a reddish brown colour with alcoholic ferric chloride. The tetramethoxy flavanone (VIII) obtained from it is colourless and does not exhibit any colour with ferric chloride but when treated with alkali it readily gets converted into the chalkone (IX). It is interesting that this flavanone (VIII) undergoes simple oxidative demethylation giving 6:7-dimethoxy-quinoflavanone (VII) as the main product. In this respect the tetramethoxy flavanone resembles the corresponding members of the flavone series (*e.g.*, calycopterin dimethyl ether) which also give rise to methoxy quinones.[5] But in the reaction with the flavanone a small quantity of pedicinin is also obtained as a result of the reaction going further involving opening of the ring and demethylation of another methoxyl group. The important point to be noted is that the quinone obtained from the tetramethoxy flavanone (VIII) is different from pedicinin and methyl pedicinin and has two methoxyl groups and thus is undoubtedly a flavanone derivative. The identity of this compound with the one obtained from the trimethyl ether (VI) resulting from despedicellin and dihydropedicinin should be taken as an additional proof of the flavanone nature of this partial methylation product and consequently of the original compounds, despedicellin and dihydropedicinin.

The action of benzoquinone on these two compounds has been next investigated. In agreement with the behaviour of similar compounds in the flavone series,[6] despedicellin does not form any sparingly soluble quinone. On the other hand, dihydropedicinin readily yields the corresponding quinone (X), with this reagent. It is a deep orange coloured crystalline solid which is quite different from pedicinin but is isomeric. It can be converted into the latter by treatment with alkali and it is therefore named allopedicinin since the name isopedicinin has earlier been employed in another connection.[2] It could also be readily methylated with diazomethane

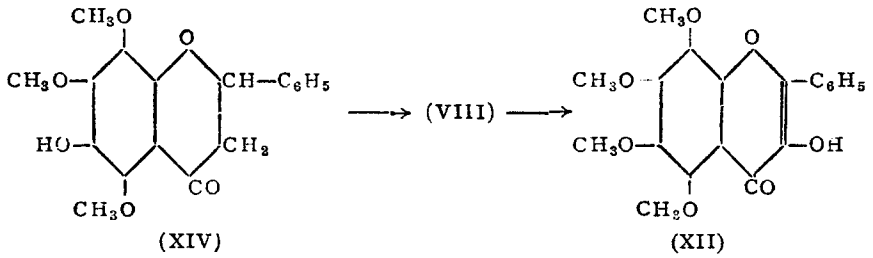
to yield a monomethyl derivative, 6:7-dimethoxy-quino-flavanone (VII) which has been already mentioned. This last mentioned quinone can further be reduced by means of sulphurous acid to the corresponding quinol (XI) and the reverse oxidation can be effected using benzoquinone. These reactions are again explicable only on the flavonone structure for these compounds.

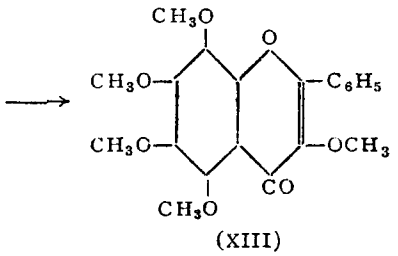


Yet another unequivocal evidence is provided by the determination of active hydrogen atoms using magnesium methyl iodide. Despedicellin gives evidence for the presence of only four hydroxyl groups and dihydropedicinin for only three. The chalkone constitution would require one more active hydrogen atom for each of them.

Since support for the flavanone structure for despedicellin and dihydropedicinin rests upon the constitution of the tetramethoxy compound (VIII) it has been felt necessary to confirm its structure as a flavanone. This has been done by converting it into the corresponding flavonol (XII) by means of isoamyl nitrite and hydrochloric acid. The 3-hydroxy compound (XII) thus obtained on methylation yields the already known pentamethyl ether (XIII) of 6:8-dihydroxy-galangin.[7]

In an earlier publication[4] it was concluded that isopedicin is 6-hydroxy-5:7:8-trimethoxy flavanone (XIV) and in support of this constitution it was pointed out that it could be obtained from pedicin by the ordinary chalkone-flavanone conversion and could be changed into pedicin easily by treatment with dilute alkali. In confirmation of this it is now shown





that it could be readily methylated with diazomethane and the product is 5:6:7:8-tetramethoxy flavanone (VIII).

Experimental

Despedicellin (II)

The following is a more convenient method of preparation than that described by Sharma and Siddiqui.[1] Pedicellin (5 g.) was dissolved in acetic anhydride (10 c.c.), hydriodic acid (20 c.c., d. 1·7) added and the mixture boiled for 1½ hours. During the later stages of the reaction a considerable amount of a crystalline solid separated out. The mixture was cooled, diluted with water and the free iodine removed by adding sodium sulphite. It was then heated to boiling, cooled and the pale yellow glistening crystals filtered. Yield 3·3 g. When recrystallised from hot alcohol, despedicellin separated out as pale yellow glistening micaceous plates melting at 255–56°. (Found: C, 55·4; H, 5·2; $C_{15}H_{12}O_6$, $2H_2O$ requires C, 55·6; H, 4·9%.)

It was sparingly soluble in alcohol and ether. When one drop of aqueous ferric chloride was added to an alcoholic solution of the substance a dark greenish blue colour was produced; the solution soon changed almost colourless and slowly developed a pale brown violet colour. The changes were markedly accelerated by the addition of another drop of the reagent. It was sparingly soluble in aqueous sodium bicarbonate. In 5% aqueous sodium carbonate it dissolved to a greenish yellow solution which changed to orange yellow. The colour reaction with caustic alkali seems to vary with the strength of the reagent and other conditions of observation. In 5% sodium hydroxide, the initial yellow colour changed on shaking to

greenish yellow and olive green which became brown red and orange and later faded slowly. When treated with *p*-benzoquinone in alcoholic solution an intense red colour was obtained which slowly faded to pale brown in the course of a few minutes. With lead acetate in alcoholic solution was formed a deep orange red precipitate which soon changed to dirty brown and later bluish green. The subsance was stable when boiled in alcoholic solution from which it can be best crystallised.

Complete methylation of despedicellin: Pedicellin

A solution of despedicellin (1 g.) in anhydrous acetone (50 c.c.) was treated with dimethyl sulphate (3 c.c.) and potassium carbonate (8 g.). After refluxing the mixture for 10 hours, the solvent was completely distilled off and the residue treated with water (50 c.c.). The mixture was cooled in ice water for 30 minutes, the undissolved white solid was filtered, washed and dried. Crystallisation from ligroin gave colourless rectangular prisms melting at 97–98°. It was identical with pedicellin.

Partial methylation of despedicellin

(i) *With diazomethane*: 5-*Hydroxy*-6 : 7 : 8-*trimethoxy flavanone* (*VI*).—A solution of despedicellin (0·8 g.) in a mixture of absolute alcohol (10 c.c.) and ether (20 c.c.) was treated with an ethereal solution of diazomethane prepared from nitrosomethyl urea (2·8 g.). The methylating agent was added in small lots with cooling in ice during 10 minutes. The initial brown colour of the solution changed to pale yellow and the reaction mixture was then kept in an ice-chest for 2 days, the container being closed with a one-holed cork carrying a capillary tube to allow for the escape of gases evolved. The solvents were completely distilled off and the residue taken up in hot ligroin. On concentrating this solution and cooling in ice-water for a few hours the methyl ether crystallised. It was purified by recrystallisation from ligroin from which it separated out in the form of pale yellow prismatic needles melting at 99–100° (Found: C, 65·7; H, 5·8, OCH_3, 28·7; $C_{18}H_{18}O_6$ requires C, 65·5; H, 5·5; methoxyl for 3 OCH_3, 28·2%). It was readily soluble in most organic solvents and sparingly in cold ligroin. In alcoholic solution it gave a stable intense green colour with ferric chloride. It was not soluble in cold aqueous sodium hydroxide but dissolved on heating to give deep yellowish brown solution.

(ii) *With dimethyl sulphate*: 2-*hydroxy*-3 : 4 : 5 : 6-*tetramethoxy chalkone* (*IX*).—A mixture of despedicellin (3 g.), dimethyl sulphate (4 c.c.), acetone (50 c.c.) and anhydrous potassium carbonate (10 g.) was refluxed for 8 hours. The solvent was distilled off and the brown residue treated with water (100 c.c.).

The mixture was extracted twice with ether and the ether extract marked (A) was shaken repeatedly with 5% sodium hydroxide. On acidifying the combined alkaline extracts with concentrated hydrochloric acid an orange yellow liquid was formed. It was extracted with ether, the ether extract dried over anhydrous sodium sulphate and distilled; 2-hydroxy-3:4:5:6-tetramethoxy chalkone separated out as a thick orange yellow oil. It did not crystallise even on keeping for a long time in the ice-chest. It was sparingly soluble in aqueous sodium hydroxide and in alcoholic solution it gave a deep reddish brown colour with ferric chloride. Yield, 1·5 g. Without further purification it was directly used for flavanone ring closure.

The alkali treated ether extract (A) on evaporation left a brown viscous semi-solid. It was digested with hot aqueous sodium hydroxide and cooled. The light brown solid was filtered from the alkaline solution and crystallised from ligroin. It formed colourless rectangular prisms melting at 97–98° and was identical with pedicellin. Yield 1 g.

Attempts were made to prepare this tetramethoxy-chalkone by the partial demethylation of pedicellin using hydrobromic acid in glacial acetic acid solution. But they were not successful. From the mixture that was formed despedicellin alone could be isolated pure.

5:6:7:8-*Tetramethoxy flavanone* (*VIII*)

A solution of 2-hydroxy-3:4:5:6-tetramethoxy chalkone prepared as described above (1 g.) was dissolved in alcohol (25 c.c.) and the solution treated with concentrated hydrochloric acid (2 c.c.) and water (10 c.c.). The mixture was gently refluxed for 24 hours and at the end of this period the alcohol was distilled off as far as possible under reduced pressure. The residue was poured into water (50 c.c.) and the mixture extracted twice with ether. The ether extract was washed with 5% aqueous sodium hydroxide twice to remove the unchanged chalkone. The extract was finally washed with water, dried over anhydrous sodium sulphate and distilled. The very pale yellow liquid product that was left behind soon became crystalline. It was recrystallised from ligroin from which it separated out in the form of colourless broad rectangular plates melting at 78–79° (Found: C, 66·4; H, 6·2; OCH_3, 35·5; $C_{19}H_{20}O_6$ requires C, 66·3; H, 5·8; 4 OCH_3, 36·0%).

It was easily soluble in most organic solvents and sparingly in ligroin. It did not give any colour with ferric chloride in alcoholic solution. It did not dissolve in cold aqueous sodium hydroxide but on heating dissolved to give a bright yellow solution. The recovered chalkone could be used again for the conversion into the flavanone.

(ii) A solution of isopedicin[4] (made from pedicin) (0·5 g.) in ether (25 c.c.) was treated with an ethereal solution of diazomethane from nitrosomethyl urea (0·5 g.). The reaction mixture was kept in the ice-chest for two days and the solvent completely distilled off. The residual pale yellow liquid was taken up in ether and shaken with dilute aqueous sodium hydroxide which removed the unchanged isopedicin. The ether solution was washed with water, dried over anhydrous sodium sulphate and distilled. The tetramethoxy flavanone left behind soon crystallised. On recrystallisation from ligroin it separated out in the form of colourless broad rectangular plates melting at 78–79° alone or in admixture with the 5:6:7:8-tetramethoxy flavanone prepared from the corresponding chalkone.

3-*Hydroxy*-5:6:7:8-*tetramethoxy flavone* (*XII*)

A solution 5:6:7:8-tetramethoxy flavanone (0·5 g.) in alcohol (20 c.c.) was heated to boiling and the hot solution was treated alternately with freshly prepared amyl nitrite (6 c.c.) and concentrated hydrochloric acid (20 c.c., d. 1·19) during the course of half an hour while keeping the mixture gently boiling. The deep red reaction mixture was set aside for two hours and poured into water (200 c.c.). After cooling in the ice-chest overnight the yellow semi-solid mass was separated by filtration, washed with water and taken up in ether. The ether solution was dried over anhydrous sodium sulphate and distilled. The yellow liquid left behind crystallised on keeping in contact with a little ether. On recrystallisation from a mixture of ether and light petroleum it separated out as glistening yellow rectangular plates melting at 131–32° (Found: C, 63·4; H, 5·5; $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0%). It was sparingly soluble in aqueous sodium hydroxide and in alcoholic solution it gave a violet brown colour with ferric chloride. Yield, 0·1 g. On acetylation it yielded the acetyl derivative which crystallised from a mixture of benzene and ligroin as colourless rectangular plates melting at 122–23°.

3:5:6:7:8-*Pentamethoxy flavone* (*XIII*)

3-Hydroxy-5:6:7:8-tetramethoxy flavone (0·1 g.) was methylated in anhydrous acetone (25 c.c.) by refluxing with dimethyl sulphate (0·3 c.c.) and potassium carbonate (2 g.) for 10 hours. The potassium salts were filtered off, washed with hot acetone and the combined acetone filtrate distilled. The residue crystallised soon on adding a few drops of water. It was recrystallised from a mixture of ether and light petroleum when it separated out as colourless rectangular plates melting at 88–89°. It was identical with 3:5:6:7:8-pentamethoxy flavone described by Seshadri and

Venkateswarlu[7] and the mixed melting point was undepressed. The sample prepared by the older method when crystallised as given above melts at this higher temperature instead of at 80–82°.

Dehydrogenation of dihydropedicinin (V) to allo-pedicinin (X)

A solution of dihydropedicinin[3] (0·5 g.) in alcohol (5 c.c.) was diluted with ether (50 c.c.) and treated with pure *p*-benzoquinone (0·5 g.). The pale yellow colour immediately changed to deep red and in a few minutes orange red shining crystals began to separate out. After keeping the reaction mixture for 3 hours at the laboratory temperature, the crystalline solid was filtered and washed with small quantities of ether. The filtrate cone tained quinhydrone and the unchanged benzoquinone mostly. The quinonon the filter (Yield, 0·35 g.) was recrystallised twice from benzene from which it separated out in the form of glistening orange red feathery rhombic plates melting at 183–84° (Found: C, 64·0; H, 4·3; $C_{16}H_{12}O_6$ requires C, 64·0; H, 4·0%). It was sparingly soluble in ether but dissolved more easily in benzene and ethyl acetate. In distilled water and alcohol it dissolved to give a stable permanganate colour. With ferric chloride in alcoholic solution the purple colour changed to brown and with lead acetate an immediate flesh-coloured precipitate was formed.

Conversion of allo-pedicinin (X) into pedicinin (III)

Allo-pedicinin prepared as described above (0·1 g.) was treated with 10% aqueous sodium hydroxide (5 c.c.). The solid readily dissolved to a purple solution which immediately changed to red. After two minutes the solution was acidified with concentrated hydrochloric acid. The orange red crystals that separated out in a few minutes were collected by filtration, washed well with water and dried. Crystallisation from benzene gave deep red rectangular plates melting at 202–3°, alone or in admixture with an authentic sample of pedicinin.

Methylation of allo-pedicinin (X) to 6: 7-dimethoxy-5: 8-quino-flavanone (VII)

A solution of allopedicinin (0·3 g.) in alcohol (10 c.c.) was treated with an ethereal solution of diazomethane prepared from nitrosomethyl urea (0·6 g.), added in small portions while cooling in ice. During the later stages of the addition an orange coloured crystalline solid separated out. The mixture was left in the ice-chest overnight. The dimethoxy quinone that separated out was filtered off and washed well with ether. The filtrate on evaporation did not give any crystalline substance. The quinone was purified by recrystallisation from ethyl acetate when it separated as glistening orange rectangular plates melting at 189–90° (Found: C, 65·4; H, 4·8;

$C_{17}H_{14}O_6$ requires C, 65·0; H, 4·5%). Yield, 0·26 g. Unlike allopedicinin, the dimethoxy quinone was not soluble in water and it did not also give any colour with alcoholic ferric chloride. It slowly dissolved in aqueous sodium hydroxide to an orange red solution.

5 : 8-*Dihydroxy*-6 : 7-*dimethoxy flavanone* (*XI*)

A solution of 6 : 7-dimethoxy-5 : 8-quino-flavanone (VII) described above (0·1 g.) in glacial acetic acid (2 c.c.) was treated with sodium sulphite (0·5 g.). On heating for a few seconds the red colour of the solution changed to brownish yellow. The solution was diluted after two minutes with water (50 c.c.) and the yellow solid that separated out was collected, filtered, washed and dried. Crystallisation from ethyl acetate gave glistening yellow rectangular prisms melting at 212–14° (Found: C, 65·0; H, 5·4; $C_{17}H_{16}O_6$ requires C, 64·6; H, 5·1%). In aqueous sodium hydroxide it readily dissolved to a deep orange red solution. With ferric chloride in alcoholic solution it gave a brown colour.

Partial methylation of dihydropedicinin (*V*) *to* 5-*hydroxy*-6 : 7 : 8-*trimethoxy flavanone* (*VI*)

Dihydropedicinin (0·4 g.) was dissolved in a mixture of alcohol (10 c.c.) and ether (20 c.c.) and the solution treated with an ether solution of diazomethane made from nitrosomethyl urea (1·2 g.). The methylation was carried out just as in the case of despedicellin. The product was taken up in ether and dried over anhydrous sodium sulphate. On evaporating the ether a pale yellow liquid was obtained which crystallised slowly on keeping in the ice-chest for some time. It was recrystallised from ligroin when it separated out in the form of very pale yellow prismatic needles melting at 99–100°. Mixed melting point with 5-hydroxy-6 : 7 : 8-trimethoxy flavanone prepared from despedicellin using diazomethane was not depressed. It was sparingly soluble in aqueous sodium hydroxide and gave an intense green colour with ferric chloride in alcoholic solution. Yield, 0·15 g.

6 : 7-*dimethoxy*-5 : 8-*quino-flavanone* (*VII*)

(*a*) *From* 5 : 6 : 7 : 8-*tetramethoxy-flavanone* (*VIII*)

A solution of 5 : 6 : 7 : 8-tetramethoxy flavanone (0·1 g.) in anhydrous ether (10 c.c.) was treated with fuming nitric acid (0·5 c.c.). The acid had to be added cautiously drop by drop while cooling the mixture in ice-water as otherwise a violent reaction resulted. On keeping stoppered for 15 minutes, the reaction mixture deposited orange red crystals of the quinone. It was filtered off and washed well with ether. The solid was macerated with

5% sodium carbonate solution for a few minutes, filtered, washed with water and recrystallised from ethyl acetate. It formed orange rectangular plates melting at 189–90° alone or in admixture with 6:7-dimethoxy-5:8-quino flavanone prepared by the methylation of allopedicinin.

(*b*) *From 5-hydroxy-6:7:8-trimethoxy flavanone (VI)*—

The above flavanone (0·1 g.) in anhydrous ether (5 c.c.) was treated with fuming nitric acid (0·2 c.c.). The product was worked up just as in the previous experiment. On crystallisation from ethyl acetate it was obtained as orange rectangular plates melting at 188–90° alone or in admixture with the sample obtained as above.

Estimation of active hydrogen

Methyl magnesium iodide was prepared in isoamyl ether solution (100 c.c.) using magnesium (6 g.) and methyl iodide (25 g.). Despedicellin and dihydropedicinin were dried in an air oven at 110–20° for 2 hours and about 0·5 g. of each was weighed out accurately and dissolved in pure anhydrous anisole (12 c.c.). The volume of methane generated by the addition of 5 c.c. of the above reagent was measured using a gas burette. (Found: Number of hydroxyl groups in despedicellin 4·12 and in dihydropedicinin 3·03. Required for the flavanone structure 4·00 and 3·00 and for the chalkone structure 5·00 and 4·00 hydroxyl groups for despedicellin and dihydropedicinin respectively.)

SUMMARY

Though despedicellin and dihydropedicinin are prepared from chalkones and were earlier given the chalkone constitution, their properties indicate that they are flavanones. This constitution is also in accordance with the recent discovery of the new effect of hydrogen bonds on the stability of flavanones. It is confirmed by the following experiments. (1) The two compounds undergo partial methylation with diazomethane yielding 5-hydroxy-6:7:8-trimethoxy-flavanone which forms with nitric acid 6:7-dimethoxy-quinoflavanone identical with the sample obtained from 5:6:7:8-tetramethoxy flavanone. (2) Dihydropedicinin undergoes oxidation with *p*-benzoquinone to form allopedicinin which is different from pedicinin. Methylation of this with diazomethane yields 6:7-dimethoxy-quinoflavanone. (3) The estimation of active hydrogen atoms agrees with the flavanone and not chalkone structure for these two compounds. That isopedicin is also a flavanone is confirmed by its methylation with diazomethane to 5:6:7:8-tetramethoxy flavanone. The constitution of the

important reference compound, tetramethoxy flavanone is established by its conversion into the corresponding flavonol.

References

1. Sharma and Siddiqui .. *J. I. C. S.*, 1939, **1**.
2. Narasimhachari and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1948, **27**, 223.
3. Salooja, Sharma and Siddiqui .. *Jour. Sci. Ind. Res.*, 1947, **6 B**, 56.
   Rao, Rao and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1948, **28**, 103.
4. Rao and Seshadri .. *Ibid.*, 1948, **27**, 375.
5. Rao, Rao and Seshadri .. *Ibid.*, 1948, **27**, 245.
6. Murty, Rao and Seshadri .. *Ibid.*, 1947, **26**, 182.
7. Seshadri and Venkateswarlu .. *Ibid.*, 1946, **23**, 192.