NUCLEAR METHYLATION OF CHALKONES AND FLAVANONES

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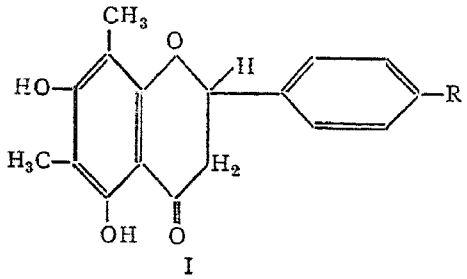
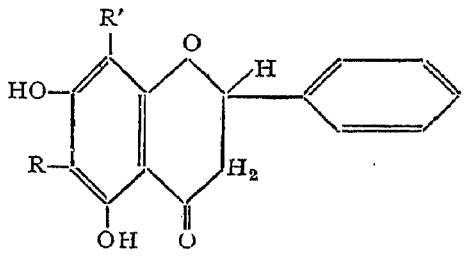
A NUMBER of flavanones with C-methyl groups are known to occur in Nature. They are matteucinol (I *a*), desmethoxy matteucinol (I *b*), farrerol (I *c*), strobopinin (II *a*) and cryptostrobin (II *b*). They have previously been synthesised by starting with nuclear methylated phloroglucinol.[1,2] They should also be capable of synthesis by direct nuclear methylation. With this object in view, a preliminary study (of simpler types) has now been made. When there is no hydroxyl in the 5-position of flavanones, the corresponding chalkones are more stable and could therefore be used for experiments on nuclear methylation. On the other hand when a 5-hydroxyl is present free, the flavanones themselves are quite stable and have to be subjected to nuclear methylation. Typical examples of these two types have now been examined under different conditions.



I

a, R=OCH_3; *b*, R=H; *c*, R=OH



II

a, R=CH_3; R′=H
b, R=H; R′=CH_3

It has been earlier established that carbonyl derivatives of resorcinol, phloroglucinol and 5:7-dihydroxy flavonoids undergo ready C-methylation in the three and six positions respectively when treated with methyl iodide and methanolic alkali.[3] When the 2:4-dihydroxy-chalkones (III) are subjected to nuclear methylation, in all cases C-methylation takes place in the 3-position just as with the corresponding acetophenones. The exact constitution of the methylation products was established by comparing them with the synthetic samples (IV) obtained by the condensation of 2-hydroxy-3-methyl-4-methoxy acetophenone (V) with benzaldehyde, anisaldehyde and veratraldehyde respectively in the presence of alcoholic potash.

1953, 1955; Ganapati and Rao, V. R., 1954; Ganapati and Rao, T. S. S., 1954) that there is a direct correlation between the abundance of some planktonic organisms and the prevailing hydrographical conditions off this coast.

The present investigation was carried out to assess the plankton production at a fixed station in the 10 fathom line off Lawson's Bay, Waltair.

MATERIAL AND METHODS

The plankton samples were collected from January to December, 1956, twice a week, between 6 and 7 A.M. A known volume of water was filtered through a fine bolting silk net of mesh size No. 30. The concentrated sample was transferred to a glass container wrapped in moist linen and the organisms examined in the living condition in the laboratory. Four such samples were taken till March and an additional fifth sample for the remaining months. Owing to the rough weather conditions the quantitative estimations for the month of May could not be worked out. Quantitative estimations were made as detailed below.

1. *Numerical estimation.*—After a preliminary examination in the living condition the sample was fixed by the addition of sufficient quantity of 40% formalin to make the total strength of dilution of the sample to about 5%. The technique of diluting and counting suggested by Nielsen (1933) was adopted and numerical estimations made by using Utermohl's inverted plankton microscope. The number of organisms in one litre of water was calculated. The macroplankton organisms were separated before counting.

2. *Volumetric estimation.*—The fixed sample was poured into a special graduated conical sedimentation funnel and the organisms allowed to settle for at least 24 hours and the volume of plankton noted.

3. *Pigment extraction.*—Harvey's method of pigment extraction (1934) was adopted using three different solvents: Acetone; Ethyl alcohol and Benzene; and Methyl alcohol. The concentration of the pigment was estimated by using a Lumetron Photoelectric Colorimeter with 420 μ filter.

4. *Total biomass.*—Bogorov's method (1934) of total Biomass estimation was employed. The fixed plankton sample was filtered through a weighed Whatman No. 42 filter-paper disc of 4·5 cm. diameter. The sample was washed with distilled water thrice to free the salt content. The total Biomass was now estimated by taking the constant dry weight of the plankton and expressed as mg./M^3 of sea-water.

5. *Total organic matter.*—Total organic matter was determined, as suggested by Riley (1938 and 1941) and Verudin (1951) by incineration of

the dried plankton and the difference between the constant dry weight, *i.e.*, total Biomass and the ash gives the total organic matter oxidised. The values are expressed as mg./M^3 of sea-water. All the weighings were made in the Metler semi-microelectric balance.

6. *Hydrography.*—Surface temperature was measured with a bulb thermometer of 0·2° C. calibration by dipping the thermometer in the water directly from the Catamaran.

Salinity:	Knudsen's method
Oxygen:	Winkler's method.
Inorganic phosphates	Denige's method as modified by Robinson and Thompson (1948).
Silicates:	Dienert and Wandenbulcke method as modified by Robinson (1948).

TABLE I

Table showing the hydrographical conditions for the year 1956

Months	Rainfall in inches	Temperature ° C.	Salinity ‰	Dissolved oxygen ml,/1.	Phosphates μg. at/1.	Silicates μg. at/1.
January ..	0·10	24·92	28·82	3·68	1·37	7·05
February ..	0·0	25·85	33·08	3·61	0·41	11·68
March ..	0·0	25·79	34·37	5·02	0·79	9·43
April ..	0·95	25·40	34·44	3·87	0·78	10·86
May ..	2·71	27·91	33·75	3·22	0·52	13·62
June ..	4·39	28·12	33·33	1·66	0·82	12·68
July ..	9·05	28·87	31·26	3·20	0·69	11·62
August ..	5·20	28·46	32·15	3·28	0·52	11·08
September ..	11·09	29·50	31·33	3·79	0·53	11·73
October ..	20·05	28·95	17·40	4·16	0·82	29·91
November ..	0·09	27·04	23·95	3·60	0·81	21·84
December ..	0·0	25·04	26·26	4·66	0·70	16·84

HYDROGRAPHICAL CONDITIONS

A series of papers (Ganapati and Murthy, 1954, 1955; Ganapati, La Fond and Bhavanarayana, 1956; La Fond, 1954, 1957; La Fond and Bhavanarayana, 1957; Bhavanarayana and La Fond, 1957; Ganapati and Subba Rao, 1957; Poornachandra Rao, 1957 and Rama Sastry and Balararamamurty, 1957) on the hydrographical conditions of the Bay of Bengal off the east coast of India with particular concentration off Waltair have appeared from the Andhra University and we have now a fairly accurate picture of the prevailing hydrographical conditions and the water movements during the different months of the year. The data collected during the year 1956 have, in the main, substantiated conditions reported for the previous years and the salient features are recorded below. The monthly average values of temperature, salinity, dissolved oxygen, phosphates and silicates are given in Table I and Fig. 1.

Temperature.—A high range of temperature was observed during the year, a maximum of 30·2° C. on 5–9–1956 and a minimum of 24·4° C. on 19–12–1956. A monthly average maximum of 29·5° C. during September and a minimum of 25·04° C. during December were observed. The low temperatures observed during March and April have been shown as due to upwelling (Ganapati and Subba Rao, 1957). The high temperatures during September and October are due to the sinking phenomenon of the surface waters (La Fond, 1954).

Salinity.—A high range of salinity was observed with a maximum of 34·88‰ on 4–4–1956 and a minimum of 13·57‰ on 8–10–1956. A monthly average maximum of 34·44‰ during April and a minimum of 17·40‰ during October were observed. The salinity of the waters is mainly influenced by the currents. The high salinity values during the northerly current period, January to June, are mainly due to incursion of the oceanic waters from the central Indian Ocean (Sewell, 1929); diminution of the flow of the rivers to the south (Sewell, 1929); and upwelling of the highly saline bottom waters (Ganapati *et al.*, 1956 and Ganapati and Subba Rao, 1957). The lowest values during October to November period are due to the dilution by large volumes of fresh-water brought by the southerly current from rivers to the north (Sewell, 1929). An inverse relationship between the salinity and silicates was observed all through the year.

Dissolved oxygen.—The dissolved oxygen content ranged from 5·67 ml./l. on 9–3–1956 to 0·33 ml./l. on 7–6–1956. The monthly average values ranged from 5·02 ml./l. during March to 1·66 ml./l. during June. A direct relationship between the phytoplankton and dissolved oxygen and

(ii) *By nuclear methylation of* 2:4-*dihydroxychalkone* (*III a*).—A solution of chalkone (2 g.) in methanolic potash (2·5 g. in 25 c.c.) was cooled in ice, treated with methyl iodide (5 c.c.) and left in a well stoppered flask in an ice bath which was allowed to assume the laboratory temperature slowly. After keeping overnight, the resulting mixture was boiled under reflux for six hours with more of methyl iodide (2 c.c.). The alcohol was distilled off under vacuum, and the residue acidified and extracted with ether. The ether concentrate was dissolved in the minimum amount of methyl alcohol and the solution cooled. The sparingly soluble fraction was crystallised thrice from methanol when it formed deep yellow rectangular rods and prisms (0·5 g.) melting at 132–33° alone or when mixed with 2-hydroxy-3-methyl-4-methoxychalkone reported above.

The methanolic mother liquor yielded a product (1·2 g.) which crystallised from ethyl acetate-petroleum ether mixture as pale yellow needles melting at 105° alone or when mixed with an authentic sample of 2-hydroxy-4-methoxychalkone.[8]

2-*Hydroxy*-3-*methyl*-4:4′-*dimethoxychalkone* (*IV b*)

(i) *By chalkone condensation.*—2-Hydroxy-3-methyl-4-methoxyaceto-phenone (1·8 g.) and anisaldehyde (1·4 g.) were condensed with alcoholic potash (3 g. in 30 c.c.) in the same way as described earlier. The product crystallised from methyl alcohol as deep yellow rods (1·4 g.) melting at 145–46° (Found: C, 72·4; H, 6·5; $C_{18}H_{18}O_4$ requires C, 72·5; H, 6·1%). It was sparingly soluble in alkali, dissolved in concentrated sulphuric acid to an orange solution and yielded a reddish brown colour with alcoholic ferric chloride.

(ii) *By nuclear methylation.*—Isoliquiritigenin (III *b*) was prepared by a modification of the method of Nadkarni and Wheeler.[6] The crude product was taken up in ether and washed with limited quantities of aqueous sodium bicarbonate in order to remove *p*-hydroxybenzoic acid, washed with water and the ether solution evaporated. The chalkone is best crystallised from a large volume of benzene, m.p. 204–05°.

Isoliquiritigenin (III *b*) (2 g.) was methylated exactly in the same way as mentioned in the case of 2:4-dihydroxychalkone and the product fractionated using methyl alcohol. The sparingly soluble fraction, after two further crystallisations from methanol, yielded 2-hydroxy-3-methyl-4:4′-dimethoxychalkone as deep yellow rectangular rods and prisms (0·5 g.) m.p. 145–46° alone or when mixed with the sample obtained by synthesis. The methanolic mother liquor yielded 2-hydroxy-4:4′-dimethoxychalkone (1·3 g.) as yellow needles,[9] m.p. 113–14°.

3-*Methyl*-2: 4: 4′-*trimethoxychalkone*:

The samples of the above chalkone (0·5 g.) prepared by the two methods were separately heated with dimethyl sulphate (0·5 c.c.) and ignited potassium carbonate (2 g.) in acetone solution for 30 hours. The product was found to be the same, 3-methyl-2: 4: 4′-trimethoxy-chalkone crystallising from ethylacetate-petroleum ether mixture as colourless stout rectangular prisms, m.p. 103–04°. It gave no ferric reaction and formed an orange solution with concentrated sulphuric acid (Found: C, 73·2; H, 6·5; $C_{19}H_{20}O_4$ requires C, 73·1; H, 6·5%).

7: 4′-*Dimethoxy*-8-*methyl-flavanone* [8-*Methyliquiritigenin dimethyl ether* (*VI*)]

2-Hydroxy-4: 4′-dimethoxy-3-methylchalkone (IV *b*) (0·1 g.) was refluxed with 4% alcoholic sulphuric acid (50 c.c., containing 10 c.c. of water) for 48 hours. Alcohol was removed under reduced pressure and water was added to the residue. The solid that separated was filtered and fractionally crystallised (five times) from alcohol when colourless needles (10 mg.), m.p. 110–11° separated. It gave no colour with alcoholic ferric chloride.

2-*Hydroxy*-3-*methyl*-4: 3′: 4′-*trimethoxychalkone* (*IV c*)

(i) *By chalkone condensation.*—2-Hydroxy-3-methyl-4-methoxy-acetophenone (1·8 g.) and veratraldehyde (1·6 g.) were condensed with alcoholic potash and the product crystallised from methyl alcohol yielding deep yellow long prismatic rods, m.p. 163–64°. It was sparingly soluble in alkali, dissolved in concentrated sulphuric acid to an orange red solution and gave a brown colour with alcoholic ferric chloride (Found: C, 69·6; H, 6·3; $C_{19}H_{20}O_5$ requires C, 69·5; H, 6·1%).

(ii) *By nuclear methylation of butein*-3′: 4′-*dimethyl ether* (*III c*).—Butein-3′: 4′-dimethyl ether (III *c*) was obtained by the method of Mauthner[10] but it was found to melt at 202–03° after crystallisation from benzene. Mauthner reported the m.p. as 127–28°, while Goschke and Tambor[11] reported its m.p. as 203°.

The above chalkone (1·75 g.) was subjected to nuclear methylation as described earlier. The sparingly soluble fraction (0·55 g.) (methanol) had m.p. 163–64° alone or when mixed with 2-hydroxy-3-methyl-4: 3′: 4′-trimethoxy chalkone (IV *c*). The mother liquor yielded butein 4: 3′: 4′-trimethyl ether,[12] m.p. 156–58°.

3-*Methyl-butein-tetramethyl ether*

This was obtained by methylation of the above chalkone (IV *c*) with dimethyl sulphate and potassium carbonate. It crystallised from ethyl

acetate-petroleum ether mixture as colourless tiny prisms, m.p. 194–95° (Found: C, 70·7; H, 6·5; $C_{20}H_{22}O_5$ requires C, 70·2; H, 6·5%).

Nuclear methylation of naringenin (VII)

Method (I): (*5-hydroxy-6-methyl-7: 4′-dimethoxy flavanone, VIII*).—Naringenin (10 g.) was dissolved in anhydrous methanol (100 c.c.) and the solution refluxed with methyl iodide (30 c.c.) for three hours during which time methanolic potash (10 g./40 c.c.) was added in ten lots and more of methyl iodide (10 c.c.) was added during the reaction to compensate for loss by vapourisation. Excess of methyl iodide and methyl alcohol were distilled off under reduced pressure and water (500 c.c.) added. The solution was acidified with cold concentrated hydrochloric acid and extracted with ether. The ether solution was extracted with saturated aqueous sodium bicarbonate, 5% sodium carbonate and 4% sodium hydroxide. The extracts yielded no appreciable product on acidification. The remaining ether solution was washed with water three times, dried over anhydrous sodium sulphate, ether distilled off and the residue crystallised from methanol, yielding colourless needles, m.p. 148° (1 g.). It gave a bluish violet colour with alcoholic ferric chloride (Found: C, 68·4; H, 6·3; $C_{18}H_{18}O_5$ requires C, 68·8; H, 5·8%).

5-Hydroxy-7: 4′-dimethoxy-6-methyl flavone (IX)

(*a*) *By selenium dioxide oxidation.*—The above C-methyl-flavanone (VIII) (0·1 g.) was dissolved in acetic anhydride (6 c.c.), selenium dioxide (0·2 g.) added and the mixture heated under reflux at 140° for five hours. The mixture was filtered off and acetic anhydride removed under reduced pressure. Water was added to the residue and the solid that separated was filtered, washed with cold water and crystallised from methyl alcohol forming pale straw coloured needles (0·05 g.), m.p. 183–85° undepressed by the nuclear methylation product of apigenin (*see* below). It gave a green colour with alcoholic ferric chloride (Found: C, 68·7; H, 4·7; $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1%).

(*b*) *By nuclear methylation of apigenin (X)*.—Apigenin (X) (2·7 g.), sodium methoxide from sodium (2·5 g.) and absolute methanol (50 c.c.) were employed following the method used earlier.[5] The method of working was, however, different and was as follows. The ether extract of the product was washed successively with saturated aqueous sodium bicarbonate (no extraction), 5% sodium carbonate (Fraction I) and 4% sodium hydroxide (Fraction II), the remaining ether solution contained neutral Fraction (III). Fraction (I) on acidification yielded a pale yellow compound, m.p. 343°

alone or mixed with apigenin. It gave a dark brown colour with alcoholic ferric chloride. Fraction (II) was acidified and the solid product crystallised from alcohol yielding a yellow compound, m.p. 270–71° undepressed by an authentic sample of apigenin-7 : 4′-dimethyl ether. It gave a dark brown colour with alcoholic ferric chloride. Neutral Fraction (III) on concentration gave a solid which crystallised from methanol as straw coloured needles, m.p. 183–85°, alone or admixed with the sample of (IX) obtained by selenium dioxide oxidation.

Method (II) (Polymethylated chalkones).—Naringenin (VII) (10 g.) was dissolved in dry methanol (100 c.c.) and methyl iodide (40 c.c.) and methanolic potash (10 g. in 40 c.c.) added all at once. The mixture was refluxed for three hours, excess of methyl iodide and methanol removed under reduced pressure and the residue treated with cold water (500 c.c.). The solution was acidified with cold concentrated hydrochloric acid and repeatedly extracted with ether. The ether solution was then successively extracted with saturated aqueous sodium bicarbonate (Fraction A), 5% sodium carbonate (Fraction B), 4% sodium hydroxide (Fraction C) and the remaining ether solution was then washed with water and dried over anhydrous sodium sulphate (neutral Fraction D).

Fraction A [5-(4′-*methyoxy-cinnamoyl*)-1 : 3 : 3-*trimethyl-cyclohexen*-(4)-ol-(4)-*dione*-(2 : 6)] (*XII a*):

The solution (200 c.c.) was acidified in the cold with concentrated hydrochloric acid. On keeping in the refrigerator overnight a yellow solid separated, which was filtered and crystallised from methanol yielding golden yellow rectangular tablets (3 g.), m.p. 177–78°, alone or when mixed with the synthetic sample described below. With alcoholic ferric chloride it gave a reddish brown colour and formed an organge yellow solution in concentrated sulphuric acid (Found: C, 69·7; H, 6·5; $C_{19}H_{21}O_5$ requires C, 69·5; H, 6·4%).

Synthesis.—5-Acetyl-1 : 3 : 3-trimethyl-cyclo-hexen-(4)-*ol*-(4)-dione-(2 : 6)[4] (XI *a*) (2·2 g.) and anisaldehyde (1·6 c.c., 1·1 mole) were dissolved in alcohol (200 c.c.) treated with aqueous caustic potash (12 g. in 10 c.c.) and kept at room temperature for forty-eight hours. It was then diluted with an equal volume of water and extracted with ether to remove excess of anisaldehyde. The aqueous solution was acidified under cooling with dilute hydrochloric acid. The mixture was extracted with ether, the extract dried over anhydrous sodium sulphate and evaporated. The residue crystallised from methanol as golden yellow rectangular tablets (0·55 g.), m.p. 177–78° undepressed by the above sample. The colour reactions were the same.

Fraction B [5-(4′-*methoxy-cinnamoyl*)-1 : 1 : 3 : 3-*tetra-methyl-cyclohexen*-(4)-ol-(4)-*dione*-(2 : 6)] (*XII b*):

The sodium carbonate extract was acidified with cold dilute hydrochloric acid and cooled in ice. The deep yellow solid product was filtered and crystallised from methanol to give yellow thin rectangular plates (3 g.), m.p. 108° alone or when mixed with a synthetic sample. It gave reddish brown colour with alcoholic ferric chloride and formed a yellow solution in concentrated sulphuric acid (Found: C, 69·2; H, 6·6; $C_{20}H_{22}O_5$ requires C, 69·7; H, 6·6%).

Synthesis.—5-Acetyl-1 :1 :3 :3-tetramethyl-cyclohexen-(4)-*ol*-(4)-dione-(2 :6)[4] (XI *b*), anisaldehyde (1·4 c.c., 1·1 mols) were condensed just as in the preparation of (XII *a*). The product crystallised from methanol to give deep yellow thin rectangular plates (0·8 g.), m.p. 108°. It gave a reddish brown colour with alcoholic ferric chloride and a yellow solution in concentrated sulphuric acid.

Neutral Fraction D [5-*Hydroxy*-6-*methyl*-7 : 4′-*dimethoxy flavanone* (*VIII*)]

The ether solution was distilled and the residue (0·2 g.) crystallised from methanol when colourless needles separated, m.p. 148° undepressed by the 5-hydroxy-6-methyl-7 : 4′-dimethoxyflavanone (VIII) sample obtained from the first method.

SUMMARY

Nuclear methylation of 2 : 4-dihydroxy-chalkones (III) give rise to 3-C-methyl derivatives just as in the case of resacetophenone. C-Methyl-chalkone-derivatives (IV) with substituents in the side phenyl nucleus have also been prepared by this method. For comparison, they have been synthesised using 3-methyl-peonol and appropriate derivatives of benzaldehyde. C-Methyl-*iso*-liquiritigenin-dimethyl-ether (IV *b*) was cyclised to the corresponding 8-methyl-flavanone (VI).

The flavanone, naringenin, when subjected to nuclear methylation under mild conditions yields 6-methyl-flavanone derivative (VIII) whereas under conditions in which the oxygen ring opens, beside the above 6-methyl flavanone (VIII), poly-C-methylated chalkones (XII a + b) are formed just as in case of phloracetophenone.

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