NUCLEAR METHYLATION OF FLAVONES AND RELATED COMPOUNDS

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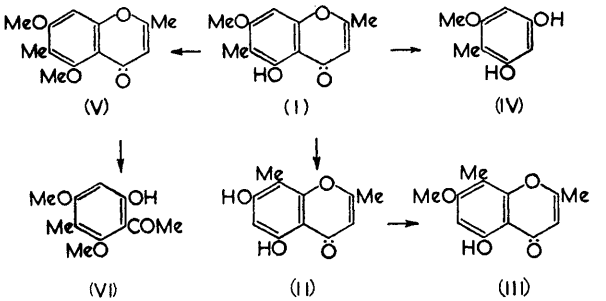
In the study of organic compounds, methylation of hydroxyl and amino-groups is a common laboratory technique, and several reagents are employed for this purpose. This process also takes place fairly freely in Nature. On the other hand, substitution of a hydrogen atom of the aromatic nucleus by a methyl group is rarer. The term, "nuclear methylation" has been used for the latter process.

Occurrence

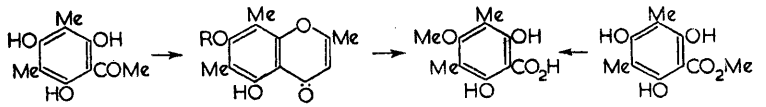
(i) *Chromones.*—Though *C*-alkylation of a benzene ring is found in a large variety of natural products, examples among chromone derivatives are comparatively few and most of these have only recently been isolated. Three such compounds, eugenitin,[1] *iso*eugenitol,[2] and *iso*eugenitin,[3] were obtained by Schmid and his co-workers from the alkali-soluble phenolic constituents of the flowers of the wild cloves of Java (*Eugenia caryophyllata*). When eugenitin was subjected to alkali fission it yielded acetone and 2-methylphloroglucinol 1-methyl ether (IV). Its methyl ether (V), on the same treatment, gave 6-hydroxy-2 : 4-dimethoxy-3-methylacetophenone (VI), showing thereby that eugenitin was 5-hydroxy-7-methoxy-2 : 6-dimethylchromone (I). When treated with hydriodic acid, eugenitin underwent simultaneous demethylation and isomerisation, forming a dihydroxy-dimethylchromone which on alkali fission yielded *C*-methylphloroglucinol and on complete methylation a methyl ether different from 5-*O*-methyl-eugenitin (V). This chromone was later found to be *iso*eugenitol (II). When subjected to partial methylation the chromone gave its 7-methyl



[1] Schmid, *Helv. Chim. Acta*, 1949, **32**, 813.
[2] Schmid and Bolleter, *ibid.*, 1949, **32**, 1358.
[3] *Idem, ibid.*, 1950, **33**, 1770.

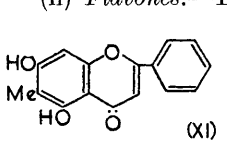
ether (III) which proved to be the same as natural *iso*eugenitin. The constitutions of these compounds have been further confirmed by synthesis by Schmid and Bolleter,[4] as well as by Mukerjee, Seshadri, and Varadarajan [5] (see below). They all contain only one *C*-methyl group in the benzene ring of the chromone either in the 6- or the 8-position.

Penfold[6] isolated a yellow crystalline substance, from the oil of *Backhousia angustifolia* Benth., which he named angustifolionol. It has been recently examined by Birch, Elliott, and Penfold.[7] They obtained acetone and



2 : 6-dihydroxy-4-methoxy-3 : 5-dimethylbenzoic acid (VIII) as its alkali-fission products which showed that angustifolionol is 5-hydroxy-7-methoxy-2 : 6 : 8-trimethylchromone (VIIa). The acid (VIII) was synthesised from methyl 2 : 4 : 6-trihydroxy-3 : 5-dimethylbenzoate (IX) by a process involving partial methylation and ester hydrolysis. The structure of angustifolionol (VIIa) has been supported by its synthesis from 3 : 5-dimethylphloracetophenone (X) by chromone condensation [to give (VIIb)] and partial methylation,[8] and also by other methods given later.

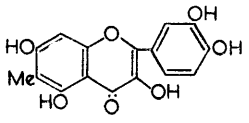
(ii) *Flavones.*—The natural flavones include only one nuclear-methylated compound, strobochrysin. This was isolated along with a number of compounds from the heart-wood of *Pinus strobus* by Lindstedt and Misiorny.[9] Its structure as 6-methylchrysin (XI) was established by alkali-degradation to *C*-methylphloroglucinol and benzoic acid, as well as by synthesis[9, 10] described below.



(XI)

(iii) *Flavonols.*—Pinoquercetin and pinomyricetin have recently been reported to occur among the components of the colouring matter of *Ponderosa* pine bark.[11] Alkali fission of their complete methylation products yielded the same ketone, identified by synthesis as 6-hydroxy-2 : 4 : ω-trimethoxy-3-methylacetophenone (XIV); in addition the former gave veratric acid, and the latter tri-*O*-methylgallic acid. Pinoquercetin is therefore 6-methylquercetin (XII), and pinomyricetin is 6-methylmyricetin (XIII). The former had earlier been synthesised by Jain and Seshadri.[12]

[4] Schmid and Bolleter, *Helv. Chim. Acta.*, 1950, **33,** p. 917.
[5] Mukerjee, Seshadri, and Varadarajan, *Proc. Indian Acad. Sci.*, 1953, **37,** *A*, 131.
[6] Penfold, *J. Proc. Roy. Soc. New South Wales*, 1923, **57,** 300.
[7] Birch, Elliott, and Penfold, *Austral. J. Chem.*, 1954, **7,** 169.
[8] Birch, Elliott, Mukerjee, Penfold, Rajagopalan, Seshadri, and Varadarajan, *ibid.*, 1955, **8,** 409.
[9] Lindstedt and Misiorny, *Acta Chem. Scand.*, 1951, **5,** 1.
[10] Mukerjee and Seshadri, *Proc. Indian Acad. Sci.*, 1953, **38,** *A*, 208.
[11] Kurth, Ramanathan, and Venkataraman, *Current Sci.*, 1955, **24,** 157; *J. Sci. Ind. Res. India*, 1956, **15,** *B*, 139.
[12] Jain and Seshadri, *J. Sci. Ind. Res., India*, 1954, **13,** *B*, 539.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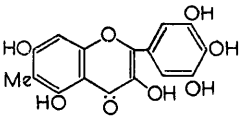
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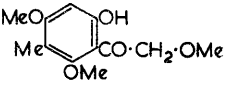
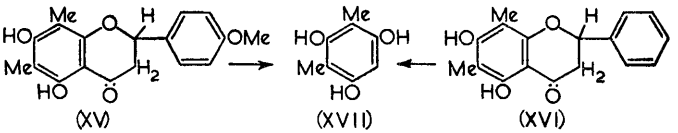

(XIV)

(iv) *Flavanones.*—Two *C*-methylated flavanones, matteucinol and demethoxymatteucinol, were quite early known to occur in Nature. They were isolated from the leaves of *Matteucia orientalis* by Munesada.[13] When fused with alkali these each gave the same phenol, 2 : 4-dimethylphloroglucinol (XVII), and in addition the first yielded 4-methoxycinnamic acid and the second cinnamic acid. Thus the structures of matteucinol and demethoxymatteucinol are 5 : 7-dihydroxy-4′-methoxy-6 : 8-dimethyl-(XV) and 5 : 7-dihydroxy-6 : 8-dimethyl-flavanone (XVI) respectively.[14] These constitutions were confirmed by synthesis from the degradation products mentioned above, using the Friedel–Crafts reaction.[15] The natural samples are optically active and their melting points are different from those of the synthetic compounds but become identical after racemisation. Matteucinol (XV) has also recently been obtained from the ethereal extract of the leaves of *Rhododendron simsii* by Arthur and Hui.[16]

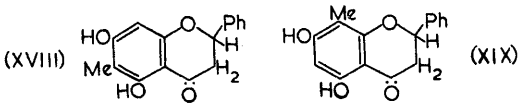

(XV) (XVII) (XVI)

Erdtman[17] isolated a new colourless compound, strobopinin, from the heartwood of *Pinus strobus* which was later found to contain an isomeric compound, cryptostrobin.[18] The former has also been found recently by Lindstedt in *Pinus monticola.*[19] With both compounds, alkali-fusion yielded 2-methylphloroglucinol and oxidation with potassium permanganate gave benzoic acid. The molecular composition ($C_{16}H_{14}O_4$) coupled with these observations indicated that these are 6- (XVIII) and 8-methyl (XIX) derivatives of 5 : 7-dihydroxyflavanone. The structures were later confirmed by synthesis involving condensation of methylphloroglucinol with cinnamoyl chloride, a mixture of the two substances being obtained in poor yield. However, the exact structure—as to which is the 6- and which the 8-methyl derivative—is still undecided.[20]

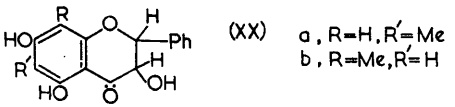
Another *C*-methylated flavanone, farrerol, has recently been reported

[13] Munesada, *J. Pharm. Soc. Japan,* 1924, **505,** 185.
[14] Fujise, *Sci. Papers Inst. Phys. Chem. Res.* (*Tokyo*), 1929, **11,** 111.
[15] Fujise and Nishi, *Ber.*, 1933, **66,** 929 ; *J. Pharm. Soc. Japan,* 1934, **55,** 1020 ; Fujise and Kubota, *Ber.*, 1934, **67,** 1905.
[16] Arthur and Hui, *J.*, 1954, 2782.
[17] Erdtman, *Svensk kem. Tidskr.*, 1944, **56,** 2.
[18] Alvarez-Nóvoa, Erdtman, and Lindstedt, *Acta Chem. Scand.*, 1950, **4,** 390.
[19] Lindstedt, *ibid.*, 1949, **3,** 1147.
[20] Lindstedt and Misiorny, *ibid.*, 1951, **5,** 11.

to occur in the leaves of *Rhododendron farrerae*; it is 4′-demethylmatteucinol (cf. XV).[20a]

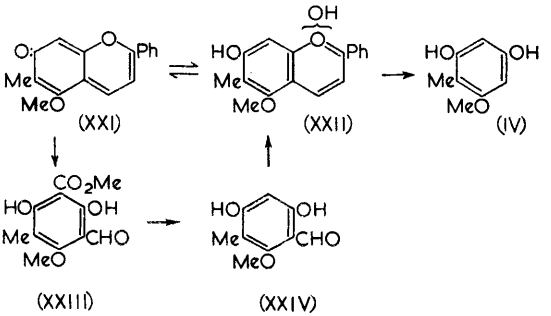


Hydroxyflavanones.—The heartwood of *Pinus strobus* yields also a colourless compound belonging to this category and it has been named strobobanksin. Alkali-fission to 2-methylphloroglucinol, oxidation with permanganate to benzoic acid, and easy dehydrogenation by palladium and cinnamic acid to a flavonol, showed it to be 3 : 5 : 7-trihydroxy-6- or -8-methylflavanone (XX a or b), though its exact constitution is unsettled.[20]



Anhydro-bases of Flavylium Salts.—Brockmann and Haase[21] isolated from " dragon's blood " resin of East Indian origin a coloured substance, dracorubin. The same compound was also obtained by Hesse[22] from the blood-red resin of the fruit-bearing parts of the palm, *Dracæna dracobluma*, along with a minor component, dracorhodin. The close relation between these two pigments was indicated by the conversion of dracorubin picrate into dracorhodin on treatment with alkali.[23]

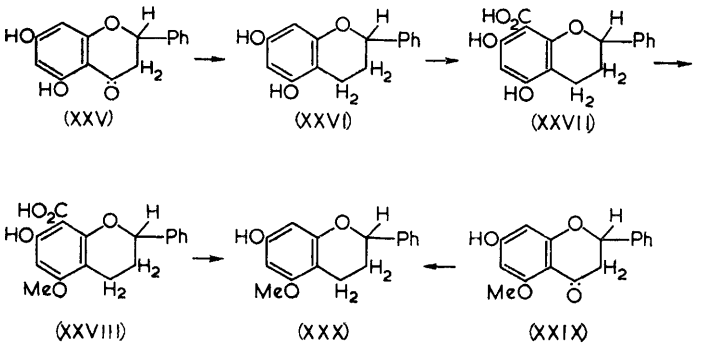
Dracorhodin, when subjected to alkali-fission, yielded acetophenone and *C*-methylphloroglucinol *β*-methyl ether. This indicated that it was the anhydro-base (XXI) of 7-hydroxy-5-methoxy-6-methylflavylium hydroxide (XXII), and this constitution was confirmed by synthesis [23, 24] from acetophenone and 4 : 6-dihydroxy-2-methoxy-3-methylbenzaldehyde (XXIV).



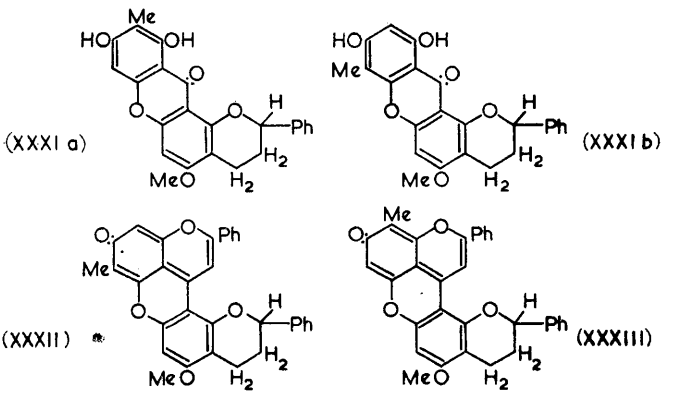
[20a] Arthur, *J.*, 1955, 3909.
[21] Brockmann and Haase, *Ber.*, 1936, **69**, 1950.
[22] Hesse, *Annalen*, 1936, **524**, 14.
[23] Brockmann and Junge, *Ber.*, 1943, **76**, 751.
[24] Robertson and Whalley, *J.*, 1950, 1882.

The aldehyde was prepared in a pure state by the hydrolysis of the anil of methyl 3-formyl-2 : 6-dihydroxy-4-methoxy-5-methylbenzoate (XXIII) with simultaneous decarboxylation of the resulting acid.[24]

Dracorubin, $C_{32}H_{24}O_5$, has a more complex structure, which has been elucidated by Robertson and Whalley.[25] On alkali-fission [26] it gave acetophenone and draconol, $C_{24}H_{20}O_6$, while oxidation with alkaline hydrogen peroxide [27] produced dracoic acid, $C_{16}H_{14}O_5$. The latter has been shown



to be 7-hydroxy-5-methoxyflavan-8-carboxylic acid (XXVIII), undergoing decarboxylation to 7-hydroxy-5-methoxyflavan (XXX) which can be obtained by Clemmensen reduction of alpinetin (XXIX). Dracoic acid itself has been synthesised from noralpinetin (XXV) as shown in the above formulæ.

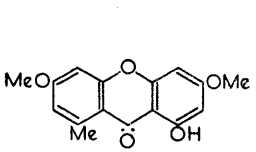


The phenol, draconol, is considered to have the structure (XXXI a or b), partly by analogy with dracorhodin (XXI) and partly because the properties of its partial methyl ether were reminiscent of a 1-hydroxyxanthone structure (resistance to fusion with alkali, formation of perchlorate,

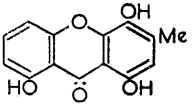
[25] Robertson and Whalley, *J.*, 1950, 1876, 3117.
[26] Brockmann and Haase, *Ber.*, 1937, **70**, 1733.
[27] Brockmann, Haase, and Freiensehner, *Ber.*, 1944, **77**, 279.

ferric reaction, colour, solubilities, and behaviour with boroacetic anhydride). Since dracorubin yields draconol (XXXI a or b) by the loss of acetophenone with the simultaneous formation of the carbonyl and 1-hydroxy-group of the xanthone residue, Robertson and Whalley [25] gave the structure of dracorubin as either (XXXII) or (XXXIII).

Other types of C-methyl-flavonoids.—Besides the above mentioned *C*-methylated benzo-4-pyrones, there are others which, though they have *C*-methyl groups in the benzene ring, do not seem to arise by nuclear methylation and so are not considered in this Review. For example, lichexanthone (XXXIV), present in the lichen, *Parmelia formosana* ; [28] ravenelin (XXXV), a metabolic product of *Helminthosporium turcium* and *H. ravenelii* ; [29] eleutherinol (XXXVI), the first natural naphthopyrone, found in *Eleuthera bulbosa* ; [30, 31] and 2-methyl-anthraquinone and -naphthaquinone derivatives occurring widely in Nature.[31a]



(XXXIV)



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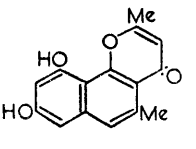
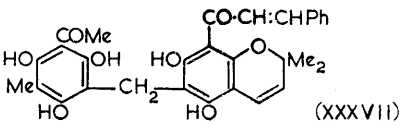


(XXXVI)

However, rottlerin [32] (XXXVII), the phenolic crystalline component of the Indian colouring matter and anthelmintic drug, " kamala " (*Mallotus philippinensis*), could be included among *C*-methylated compounds. It is a chalcone derivative having a *C*-methylphloracetophenone unit. Here *C*-methylation is in the substituent group and not in the main chalcone skeleton.



(XXXVII)

Synthesis

(i) Methylation with methyl iodide and methanolic potassium hydroxide was the earliest method for the preparation of nuclear methylated flavon-

[28] Asahina and Nagami, *Bull. Chem. Soc. Japan*, 1942, **17**, 202; Aghoramurthy and Seshadri, *J. Sci. Ind. Res., India*, 1953, **12**, *B*, 73, 350.

[29] Raistrick, Robinson, and White, *Biochem. J.*, 1936, **30**, 1303.

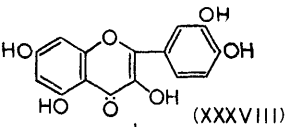
[30] Schmid, Ebnother, and Meijer, *Helv. Chim. Acta*, 1950, **33**, 1751 ; 1952, **35**, 910, 928.

[31] Birch and Donovan, *Austral. J. Chem.*, 1953, **6**, 373.

[31a] Aghoramurthy and Seshadri, *J. Sci. Ind. Res., India*, 1954, **13**, *A*, 114.

[32] Perkin and Perkin, *Ber.*, 1886, **19**, 3109 ; Hummel and Perkin, *J. Soc. Chem. Ind.*, 1895, **14**, 460 ; Perkin, *ibid.*, 1900, **19**, 519 ; McGookin and Robertson, *J.*, 1937, 748 ; 1938, 309 ; 1939, 1579, 1587 ; 1948, 113 ; Brockmann and Maier, *Annalen*, 1938, **535**, 149.

oids.[33–37] Sodium methoxide can also be used in place of potassium hydroxide,[38] and sometimes has a marked advantage. This method invariably leads to the production of a mixture containing the simple methyl ethers (partial as well as complete) along with the methyl ethers of nuclear methylated products. But the separation of *C*-methyl compounds is not very difficult because of their markedly lower solubility in organic solvents. For example, quercetin (XXXVIII) generally yields a mixture of 3 : 7 : 3′ : 4′-tetramethyl ethers of quercetin (XXXIXa) and 6-methylquercetin (XL), as well as quercetin pentamethyl ether (XXXIXb). Of all these, the *C*-methylated compound is the most sparingly soluble in ether as well as in methyl alcohol, and can thus be easily separated.[12, 37]



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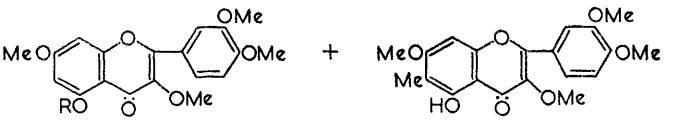
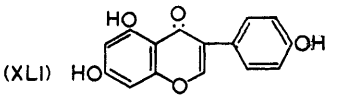
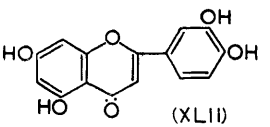


(XXXIX) a, R=H; b, R=Me (XL)

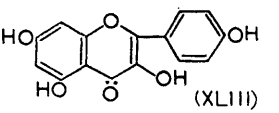
Though it was known even towards the end of the last century that nuclear methylation of genistein[33, 34] (XLI), luteolin[34] (XLII), and kæmpferol [35] (XLIII) took place in the fused benzene ring [alkali-fission yielded *C*-methylphloroglucinol *β*-methyl ether (IV)], the exact location of the *C*-methyl group could not be established. It is only recently that a number of chromone derivatives of definite constitution have been synthetically obtained (see p. 178) and, by comparison with them, the nuclear methylation products have been uniformly shown to be 6-methyl derivatives (see ref. 39).



(XLI)



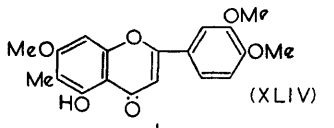
(XLII)



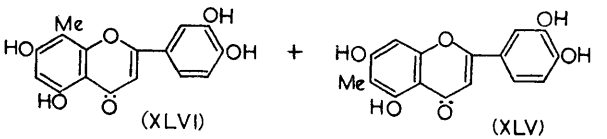
(XLIII)

[33] Perkin and Newbury, *J.*, 1899, **75,** 836.
[34] Perkin and Horsfall, *J.*, 1900, **77,** 1311, 1317.
[35] Ciamician and Silber, *Ber.*, 1899, **32,** 861.
[36] Waliaschko, *Arch. Pharm.*, 1909, **247,** 453.
[37] Perkin, *J.*, 1913, **103,** 1635.
[38] Baker and Robinson, *J.*, 1926, 2713.
[39] Jain and Seshadri, *J. Sci. Ind. Res., India*, 1955, **14,** *A*, 227, for collected references.

It was earlier considered that the demethylation of these *C*-methylated methyl ethers with hydriodic acid yielded the corresponding hydroxy-6-methyl compounds,[34] but it is now known that this is not invariably so. With simple chromone and flavone derivatives, a mixture results owing to partial isomeric change during demethylation and the isomers have to be separated.[5, 10, 40] For example, 6-methyl-luteolin 7 : 3′ : 4′-trimethyl ether (XLIV) gives a mixture of 6-methyl-luteolin (XLV) and 8-methyl-luteolin (XLVI).[40] Under ordinary conditions, chromonols, flavonols,[12, 41, 42] and *iso*flavones [43–45] do not suffer this isomeric change. But it is possible that under drastic conditions even these can be made to do so.[46] If instead of hydriodic acid, aluminium chloride in dry benzene is used for demethylation, in most cases there is little isomeric change ; even here under more drastic conditions, a change can be brought about.[47] This subject of ring isomeric change in flavonoids has been recently reviewed by Mukerjee and Seshadri.[48]



(XLIV)

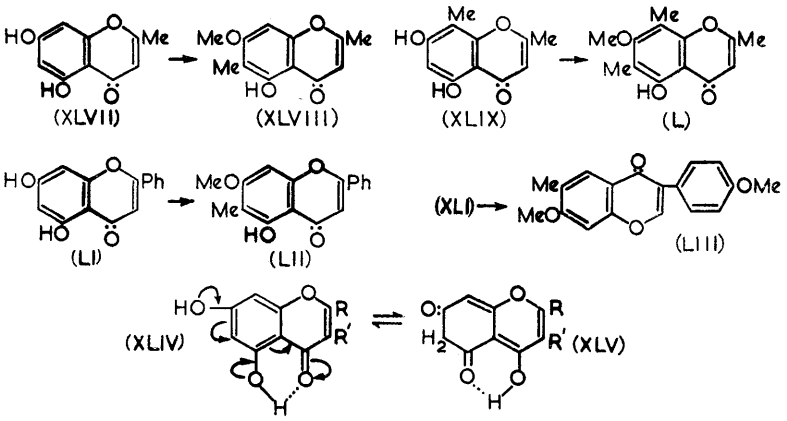


(XLVI) (XLV)

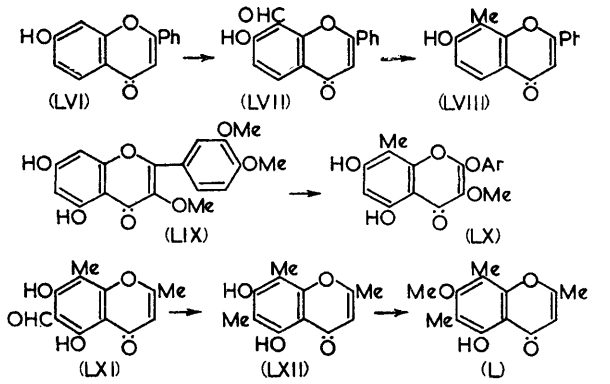
Nuclear methylation can be achieved satisfactorily with 5 : 7-dihydroxychromones. For example, noreugenin (XLVII) forms eugenitin [10, 49] (XLVIII) ; *iso*eugenitol (XLIX) gives angustifolionol [8] (L) ; chrysin (LI) gives strobochrysin 7-methyl ether [10] (LII) ; quercetin (XXXVIII) gives pinoquercetin 3 : 7 : 3′ : 4′-tetramethyl ether [12] (XL) (see p. 175) ; and genistein (XLI) gives 6-methylgenistein 7 : 4′-dimethyl ether [33, 34, 38, 50] (LIII). However, the method is not suitable for the nuclear methylation of the simpler 7-hydroxychromone derivatives.[41] The reasons for this, as well as the mechanism of nuclear methylation, have been explained by Jain

[40] Bannerjee and Seshadri, *J. Sci. Ind. Res., India*, 1954, **13,** *B*, 598.
[41] Jain and Seshadri, *Proc. Indian Acad. Sci.*, 1954, **40,** *A*, 249.
[42] *Idem*, *J. Sci. Ind. Res., India*, 1953, **12,** *B*, 564.
[43] Seshadri and Varadarajan, *Proc. Indian Acad. Sci.*, 1953, **37,** *A*, 145, 508, 514, 526.
[44] Whalley, *J. Amer. Chem. Soc.*, 1953, **75,** 1059.
[45] Iengar, Mehta, Seshadri, and Varadarajan, *J. Sci. Ind. Res., India*, 1954, **13,** *B*, 166.
[46] Donnelly, Philbin, and Wheeler, *Chem. and Ind.*, 1954, 163 ; Baker, Dunstan, Harborne, Ollis, and Winter, *ibid.*, 1953, 277.
[47] Whalley, *Chem. and Ind.*, 1953, 277 ; *J.*, 1953, 3366.
[48] Mukerjee and Seshadri, *Chem. and Ind.*, 955, 271.
[49] Whalley, *J. Amer. Chem. Soc.*, 1952, **74,** 794.
[50] Mehta and Seshadri, *J.*, 1954, 3823.

and Seshadri.[39] It has been shown that the minimum requirement for nuclear methylation is the existence of a resorcinol-carbonyl unit (LIV) capable of undergoing tautomeric change into a β-diketonic structure (LV), which is the reactive structure for nuclear methylation.



(ii) The second method of nuclear methylation is a two-stage process, consisting of an aldehyde synthesis followed by reduction. The method most commonly used for the first stage is a Duff reaction, which introduces an aldehyde group into the reactive 8-position and, if this is not available, into the 6-position. The second stage involves catalytic reduction with the calculated amount of hydrogen. This method is very satisfactory for the preparation of 8-methyl derivatives of 5 : 7-dihydroxy- and 7-hydroxy-flavonoids. It was applied earlier in simple cases [*e.g.*, conversion of

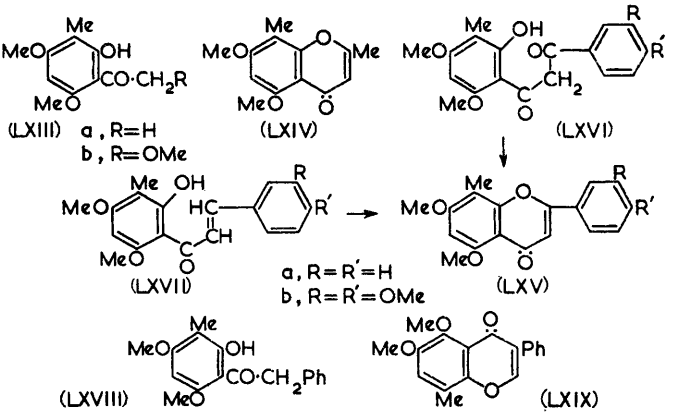


7-hydroxyflavone (LVI) into its 8-methyl derivative (LVIII) through the intermediate aldehyde (LVII)] by Rangaswami and Seshadri [51] and has recently been used by others for the preparation of 8-methylquercetin 3 : 3′ : 4′-trimethyl ether (LX) from quercetin trimethyl ether [12] (LIX), and

[51] Rangaswami and Seshadri, *Proc. Indian Acad. Sci.*, 1939, **9**, *A*, 7.

also for the synthesis of angustifolionol (L) through the stages (LXI) and (LXII).[8]

(iii) An unambiguous method for the synthesis of 8-methyl derivatives is to employ 2-hydroxy-4 : 6-dimethoxy-3-methylacetophenone (LXIIIa) or its ω-methoxy-derivative (LXIIIb) for chromone and flavone condensations, yielding 5 : 7-dimethoxy-8-methyl compounds. For example, the ketone (LXIIIa), on ring closure with sodium acetate and acetic anhydride, yields 5 : 7-dimethoxy-2 : 8-dimethylchromone (*iso*eugenitin monomethyl ether) [4, 5] (LXIV). The corresponding flavone [8-methylchrysin dimethyl ether (LXVa)] and the ether (LXVb) have been prepared in two ways : by preparation of the diketone (LXVI) and its cyclisation,[10, 40] and by the preparation of the chalcone (LXVII) and its oxidation with selenium dioxide.[9, 40] For flavonols, Allan–Robinson condensation and chalcone condensation followed by oxidation with selenium dioxide are satisfactory.[12, 41, 42, 52] For the preparation of 8-methyl*iso*flavone derivatives

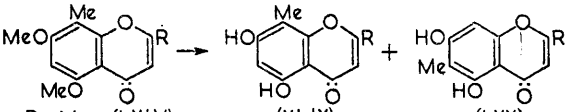


(LXIX), benzyl 2-hydroxy-4 : 6-dimethoxy-3-methylphenyl ketone (LXVIII) with the required substituents in the phenyl group has been subjected to the usual methods of *iso*flavone ring closure, *viz.*, heating with acetic anhydride and fused sodium acetate or condensation with sodium and ethyl formate.[43, 45, 53]

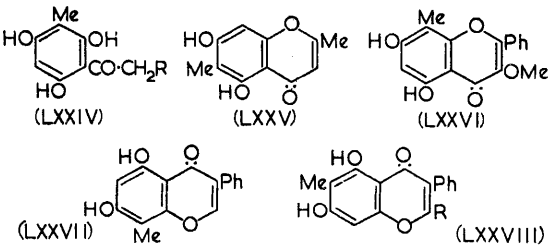
When 8-methylflavonol and *iso*flavone methyl ethers having 3-methoxy- and 3-phenyl substituents respectively are demethylated by hydriodic acid under ordinary conditions, they do not undergo isomeric change, but only demethylation. On the other hand, 8-methylchromone and flavone methyl ethers [*e.g.*, 5 : 7-dimethoxy-2 : 8-dimethylchromone [54] (LXIV) and 8-methylchrysin dimethyl ether [5] (LXXI)] give mixtures of hydroxy-6- and -8-methyl-chromones and -flavones [eugenitol (LXX) and *iso*eugenitol (XLIX) ; 8- (LXXII) and 6-methylchrysin (LXXIII)]. The proportions,

[52] Lindstedt and Misiorny, *Acta Chem. Scand.*, 1951, **5**, 1213.
[53] Karmarkar, Shah, and Venkataraman, *Proc. Indian Acad. Sci.*, 1952, **36**, *A*, 552.
[54] Mukerjee and Seshadri, *Chem. and Ind.*, 1955, 1009.

however, vary ; in chromones the 8-methyl derivative is predominantly the major product while in flavones both are obtained in almost equal quantities. The isomerisation is avoided by demethylation with aluminium chloride in benzene under ordinary conditions.[10, 40]



(iv) If, in the above synthesis, the hydroxy-ketones (LXXIV ; R = H, OMe, Ph, or substituted phenyl group) are employed instead of the methyl ethers, a mixture is obtained containing the 6- and 8-methyl derivatives of the hydroxy-chromones, -flavones, etc. The proportion of the isomers varies from case to case. In simple chromone condensations, the 6-methyl compound (eugenitol) (LXXV) constitutes the major product [10] and the 8-methyl isomer (XLIX) the minor ; [54] in flavones these are in almost equal amount ; [10, 40, 45] in flavonols, the major product (75%) is the 8-methyl compound [12, 41, 42] (LXXVI) ; and in *iso*flavones, when a high-temperature reaction such as heating with acetic anhydride and fused sodium acetate is used, the products are almost completely 8-methyl compounds (LXXVII), whereas in the low-temperature reactions [55] (ethoxalyl chloride or acetyl chloride in pyridine at 0° being used) 6-methyl derivatives (LXXVIII) are the most prominent products.[50] Obviously the comparative reactivity of the hydroxyl groups situated *ortho* or *para* to the *C*-methyl group in the ketone used for ring closure depends on the temperature and other conditions.



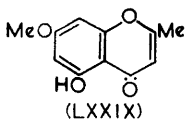
Biogenesis

Though considerable thought has been given to biological methylation and useful experimental results have been obtained, we are still far from having information adequate for us to work out precise details of biogenesis. Consequently, any mechanism suggested has to be tentative and in the nature of a working hypothesis.

In discussing the biogenesis of nuclear-methylated flavonoids, the following points have to be considered. A number of these compounds carry a 6-methyl group [eugenitin (I), strobochrysin (XI), and dracorhodin (XXI)].

[55] Baker, Harborne, and Ollis, *J.*, 1953, 1860.

However, there are representatives [*iso*eugenitol (II) and *iso*eugenitin (III)] which have an 8-methyl group, and examples containing methyl groups in both the 6- and the 8-position [matteucinol (XVa), farrerol, demethoxymatteucinol (XVI), and angustifolionol (VIIa)]. In a number of cases, the accompanying hydroxyl groups have undergone partial methylation [eugenitin (I), *iso*eugenitin (III), and dracorhodin (XXI)], and there are others in which no *O*-methylation has taken place [strobochrysin (XI), pinoquercetin (XII), and pinomyricetin (XIII)]. Complete *O*-methylation is not found in any of these. Further, in a large number of plant sources *C*-methyl compounds occur along with simpler compounds, not containing the *C*-methyl group. For example, in the flowers of *Eugenia caryophyllata*, eugenin (LXXIX) has no *C*-methyl group in the benzene ring, whereas three other components mentioned earlier (I, II, and III) contain either a 6- or an 8-methyl group. Similarly, in the heartwood of *Pinus strobus*, strobochrysin (XI) and chrysin (LI) occur together.



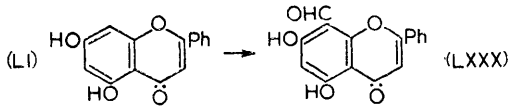
(LXXIX)

From the above facts, it seems that *C*-methylation takes place after the main carbon skeleton is formed but before *O*-methylation. The role of formaldehyde or its equivalent as biological methylating agent has been studied for a considerable time and has been discussed in two recent reviews, one by Geissman and Hinreiner [56] and the other by Challenger.[57] A point which strongly supports this hypothesis was brought out by Seshadri [58] in connection with the biogenesis of lichen acids. It is the occurrence of a single carbon atom in all states of oxidation, —CH_3, —CH_2·OH, —CHO, and —CO_2H, which is readily explained as arising from the initial —CH_2·OH group formed by the condensation of formaldehyde and undergoing reduction to CH_3 and oxidation to the other groups. An alternative may be that transmethylating agents provide methyl groups; this is also discussed in the above-mentioned reviews. Both reagents are electrophilic (cationoid), but they may react in different ways and under different conditions.

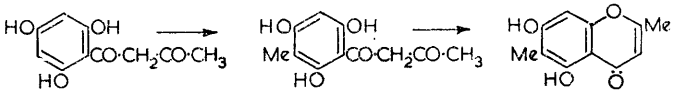
The following two possibilities can be envisaged for the formation of *C*-monomethyl compounds. The first would be *C*-methylation of the fully formed 5 : 7-dihydroxyflavonoids. The predominantly reactive position seems to be position 8, and this conclusion is supported by the conversion of chrysin (LI) into chrysin-8-aldehyde [59] (LXXX) and more recently of quercetin 3 : 3′ : 4′-trimethyl ether (LIX) into the 8-aldehyde and then into the 8-methyl quercetin derivative [12] (LX) (see p. 177). However, experiments on nuclear methylation by means of methyl iodide [39] indicate that the 6-position is predominently reactive with this reagent, and this has been explained as due to the capacity of the system to react in the isomeric diketonic form (see p. 177). This methylation is probably analogous to the action of transmethylating agents. Even with formaldehyde or its equivalent, if the 8-position is protected by some means, say, enzymic

[56] Geissman and Hinreiner, *Bot. Rev.*, 1952, **18**, 77.
[57] Challenger, *Quart. Rev.*, 1955, **9**, 255.
[58] Seshadri, *Proc. Indian Acad. Sci.*, 1944, **20**, *A*, 1.
[59] Seshadri and Varadarajan, *ibid.*, 1949, **30**, *A*, 342.

adsorption, the 6-position can function. This is suggested by the synthesis of angustifolionol (VIIa) from *iso*eugenitol [8] (XLIX), even by the aldehyde method (see pp. 177, 178). Hence the fully formed pyrone derivative can undergo nuclear methylation either in the 8- or in the 6-position by the mechanisms available in plants.



The second possibility would be *C*-methylation prior to ring-closure. In connection with the biogenesis of the chromones of *Eugenia caryophyllata*, Mukerjee *et al.*[5] suggested that the 8-methyl group arose directly by the nuclear methylation of the 5 : 7-dihydroxychromone by means of the formaldehyde equivalent, whereas the 6-methyl compound was the result of the nuclear methylation at the earlier diketonic stage, noreugenone (LXXXI), subsequent ring closure giving preferentially the 6-methyl derivative (LXXXII). This was based on the general results of pyrone ring-closure available at that time But more recently it has been shown that both 6- and 8-methyl compounds are produced simultaneously in varying proportions [54] under the same conditions, and this supports Schmid and Bolleter's suggestion [2] that eugenitin (I) and *iso*eugenitin (III) arise from the same precursor, by ring closure in the two possible ways.



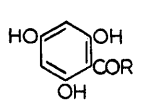
(LXXI) (LXXXII)

For evolution of 6 : 8-dimethyl compounds such as matteucinol (XV), farrerol demethoxymatteucinol (XVI), and angustifolionol (VIIa), two routes are possible : (i) methylation in the 8-position of the 6-methyl compound ; and (ii) methylation in the 6-position of the 8-methyl compound. Both these are possible in Nature. In the laboratory, the aldehyde method works satisfactorily in both cases [8] (see p. 177), but the method using methyl iodide is applicable only to the 8-methyl compound since the new methyl group always enters the 6-position. Thus, angustifolionol is readily formed by methylation of *iso*eugenitol (XLIX) and not of eugenitol [8] (LXXXII).

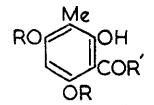
A further possibility is dialkylation at an earlier stage, before the chromone ring is closed. Definite information has not been available on this reaction. The action of formaldehyde is known to be complex, involving more than one molecule of phloroglucinol.[60] Monoalkylation of phloracetophenone [61] (LXXXIIIa) and phloro*iso*butyrophenone [62] (LXXXIIIb) with methyl iodide by the potassium carbonate–acetone method is fairly easy. The former yields 2-hydroxy-4 : 6-dimethoxy-3-methylacetophenone

[60] Boehm, *Annalen*, 1903, **329**, 269.
[61] Curd and Robertson, *J.*, 1933, 457.
[62] Hems and Todd, *J.*, 1940, 1208.

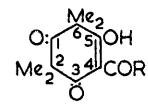
(LXXXIVa), and the latter forms baeckeol (LXXXIVc) (2-hydroxy-4 : 6-dimethoxy-3-methyl*iso*butyrophenone) which is a phenolic constituent of the essential oils from certain species of *Myrtaceæ*.[63] Further *C*-methylation does not take place under these conditions.[61] Jain and Seshadri [64] therefore subjected *C*-methylphloracetophenone (LXXXIVb) to methylation under conditions found to be the most satisfactory for resacetophenone ; excess of both methyl iodide and methanolic alkali were added in one lot, and the mixture was refluxed for several hours. It was however found to yield a *C*-polymethylated product, 4-acetyl-5-hydroxy-2 : 2 : 6 : 6-tetramethyl-*cyclo*hex-4-ene-1 : 3-dione (LXXXVa). The same product was also obtained directly from phloracetophenone (LXXXIIIa). An analogous structure is found in leptospermone (LXXXVb) which occurs in the oil of *Leptospermum flavescens* : [65, 66] it was obtained from phloro*iso*valerophenone (LXXXIIIc) by the above method in good yields.[64, 66]



(LXXXIII) a, R=Me
b, R=Pr^{i}
c, R=Bu^{i}
d, R=H



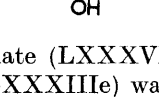
(LXXXIV) a, R=R′=Me
b, R=H, R′=Me
c, R=Me, R′=Pr^{i}

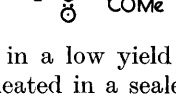


(LXXXV) a, R=Me
b, R=Bu^{i}
c, R=H

Therefore, in subsequent experiments, restricted quantities of alkali (one, two, and three equivalents) were used, always with an excess of methyl iodide, in order to avoid strongly alkaline conditions.[64] 3 : 5-Dimethylphloracetophenone (LXXXVIa) could be obtained only in poor yields. The monomethylphloracetophenone (LXXXIVb) is comparatively easy to obtain in satisfactory yields, and similarly the higher methylation products (tri- and tetra-) (see also Riedl and Risse [67]). It therefore appears that the dimethyl compound is rapidly consumed in further reaction. Another possibility, suggested by Riedl and Risse,[67] is the formation of a *gem.*-dimethyl derivative, *e.g.*, 5-acetylfilicinic acid (LXXXVII), competing with the formation of the 3 : 5-dimethyl compound. These conclusions are further supported by two earlier observations, (*a*) formation of methyl 3 : 5-dimethyl-

(LXXXVI)
a, R = Me
b, R = H
c, R = OMe





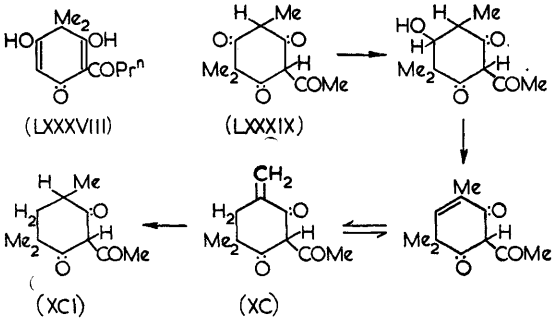
(LXXXVII)

phloroglucinolcarboxylate (LXXXVIc) in a low yield when silver phloroglucinolcarboxylate (LXXXIIIe) was heated in a sealed tube with methyl

[63] Penfold *et al.*, *J. Proc. Roy. Soc., New South Wales*, 1922, **56**, 87 ; 1925, **59**, 351 ; 1936, **71**, 291.
[64] Jain and Seshadri, *Proc. Indian Acad. Sci.*, 1955, **42**, *A*, 279.
[65] Penfold, *J. Proc. Roy. Soc., New South Wales*, 1921, **55**, 51.
[66] Briggs *et al.*, *J.*, 1938, 1193 ; 1945, 706 ; 1948, 383.
[67] Riedl and Risse, *Annalen*, 1954, **585**, 209.

iodide,[68] and (*b*) the formation of 4-formyl-5-hydroxy-2 : 2 : 6 : 6-tetramethyl-*cyclo*hex-4-ene-1 : 3-dione (LXXXVc) from formyl-3 : 5-dimethylphloroglucinol (LXXXVIb) by treatment with methyl iodide and methanolic potassium hydroxide.[69]

That carbonyl derivatives of phloroglucinol readily undergo *C*-polymethylation even in Nature is borne out by the occurrence of a large number of *C*-polymethylated ketones. Important examples are leptospermone (LXXXVb) and butyrylfilicinic acid [70] (LXXXVIII). Angustione (XCI) and dehydroangustione (XC or its tautomer), occurring in the oil of *Backhousia angustifolia*,[71] could also come under this category if stages (*a*) reduction of a keto-group, (*b*) dehydration, and (*c*) further reduction of a double bond are envisaged, as in (LXXXIX) to (XCI).[64]



A number of compounds found in male and female ferns have two such nuclear methylated phloroglucinol units, *e.g.*, aspidin [70] (XCII), albaspidin [72] (XCIII), and flavaspidic acid [69, 72] (XCIV). Some compounds, such as protokosin (XCV), α-kosin (XCVIa), and β-kosin (XCVIb), present in the anthelmintic drug, kousso,[73] and ψ-aspidin [71] (XCVII), are in lower states of methylation. Aspidinol (XCVIII) is a *C*-monomethyl derivative of phlorobutyrophenone, also found in ferns,[74] and usnic acid (XCIX) is a unique example in which two *C*-methylphloracetophenone units have combined to yield a dibenzofuran derivative.[75]

A survey of the above-mentioned compounds supports the view that,

[68] Herzig, Wenzel, and Altmann, *Monatsh.*, 1901, **22**, 219.

[69] Herzig and Wenzel, *ibid.*, 1905, **26**, 1366.

[70] Boehm, *Annalen*, 1898, **302**, 171 ; 1899, **307**, 250 ; 1901, **318**, 230 ; 1903, **329**, 321.

[71] Gibson, Penfold, and Simonsen, *J.*, 1930, 1184 ; Cahn, Gibson, Penfold, and Robinson, *J.*, 1931, 286 ; Birch, *J.*, 1951, 3026 ; Ensor and Wilson, *Chem. and Ind.*, 1955, 1010 ; Chan and Hassall, *J.*, 1955, 2860 ; Birch and Elliott, *Chem. and Ind.*, 1956, 124 ; *Austral. J. Chem.*, 1956, **9**, 95.

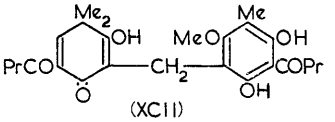
[72] Robertson and Sandrock, *J.*, 1933, 1617 ; McGookin, Robertson, and Simpson, *J.*, 1953, 1828 ; Riedl, *Annalen*, 1954, **585**, 32.

[73] Hems and Todd, *J.*, 1937, 562 ; Birch and Todd, *J.*, 1952, 3102.

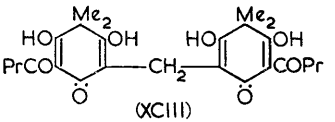
[74] Karrer and Widmer, *Helv. Chim. Acta*, 1920, **3**, 392 ; Robertson and Sandrock, *J.*, 1933, 819.

[75] Curd and Robertson, *J.*, 1937, 898 ; Barton, Deflorin, and Edwards, *Chem. and Ind.*, 1955, 1039 ; *J.*, 1956, 530 ; Schöpf and Ross, *Naturwiss.*, 1938, **47**, 772 ; *Annalen*, 1941, **546**, 1.

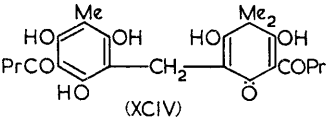
in general, single methyl groups enter the 3- and the 5-position of the phloroglucinol nucleus at an early stage, and that the *gem.*-dimethyl groups are formed by further methylation. Isolation, though in poor yield, of



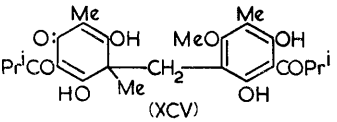
(XCII)



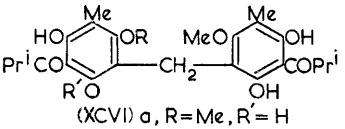
(XCIII)



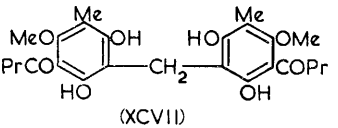
(XCIV)



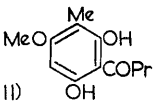
(XCV)



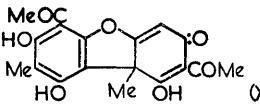
(XCVI) a, R=Me, R'=H
b, R=H, R'=Me



(XCVII)



(XCVIII)



(XCIX)

3 : 5-dimethylphloracetophenone (LXXXVIa) during nuclear methylation of phloracetophenone [64, 67] therefore indicates the possibility of dialkylation before pyrone ring-closure in the evolution of 6 : 8-dimethylchromone derivatives.