Tetrahedron. Vol. 26, pp. 1409–1416. Pergamon Press 1970. Printed in Great Britain

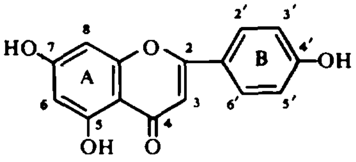
OXIDATIVE COUPLING OF APIGENIN

R. J. MOLYNEUX[1], A. C. WAISS, JR. and W. F. HADDON
Western Regional Research Laboratory, Agricultural Research Service,
U.S. Department of Agriculture, Albany, California 94710

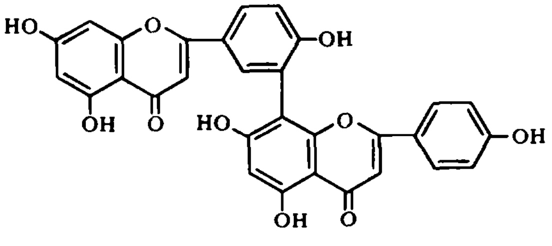
(*Received in USA* 5 *September* 1969; *Received in the UK for publication* 18 *November* 1969)

Abstract—Two novel biflavonyls have been prepared by phenolic oxidative coupling of apigenin, using alkaline potassium ferricyanide as the oxidizing agent. The structures of these dimers have been established as 3,3- and 3,3′-biapigeninyl respectively, primarily by spectroscopic methods. The synthetic compounds differ from all known natural biflavonyls in that linkage through the A ring of at least one of the flavonoid moieties is not observed. This indicates that the bioxynthesis of the natural compounds probably does not involve direct radical coupling.

THE oxidative coupling of free radical species derived from phenolic substrates is now widely accepted as the pathway by which many complex natural products are biosynthesized.[2,3] Such a route has been suggested as being involved in the formation of the group of compounds known as the biflavonyls, occurring widely distributed among the Gymnosperms, which possess the apigenin moiety (I) as a common structural feature.[4] Thus the parent biflavonyls, amentoflavone (II),[5] cupressuflavone (III)[6] and hinokiflavone (IV),[7] together with various O-methyl ethers, exhibit either C—C or C—O bonding between the flavonoid units which might be expected to arise



I



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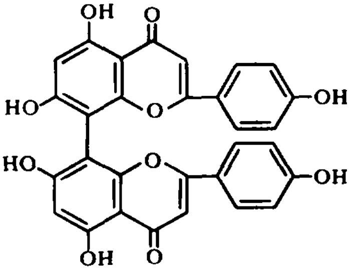
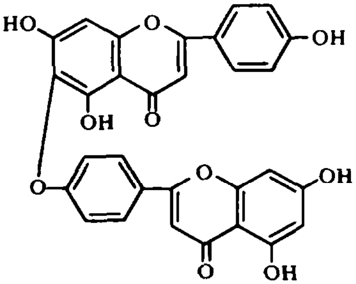


III



IV

through oxidative coupling of an apigenin-derived radical by three of the many modes of dimerization theoretically possible.

In view of the success which has been achieved during recent years in synthesizing natural products by oxidative coupling of phenolic monomers using one-electron oxidizing agents, it seemed appropriate to attempt the synthesis of biflavonyls by such a method and to determine whether the resulting product or products would exhibit the same modes of coupling as those occurring in the natural series of compounds.

Oxidation of apigenin in aqueous sodium carbonate solution at room temperature under an atmosphere of nitrogen with potassium ferricyanide yielded a product which was shown by thin-layer chromatography on silica gel in toluene-ethyl formate-formic acid (5:4:1) to be a mixture of unreacted apigenin and two compounds with R_f values similar to those of amentoflavone and hinokiflavone.[8]

After removal of most of the apigenin by trituration with cold methanol, the less soluble residue was subjected to chromatography on Sephadex LH-20 in methanol, the eluate being monitored by its UV absorption at 254 mμ. In this manner the reaction product was separated into three fractions, consisting of apigenin, dimer A (11%) and dimer B (21%)* respectively, in order of elution. Each of the fractions gave a single spot on TLC. It is of interest to note that although Sephadex LH-20 is normally regarded as a gel filtration medium, the apigenin was found to precede the dimeric fractions on elution and separation must therefore be achieved through differential adsorption, further demonstrating the effectiveness of this material for column chromatography of flavonoid compounds.[9]

Dimer A crystallized from wet ethyl acetate as yellow plates, m.p. 315–317°, analyzing for $C_{30}H_{18}O_{10}\cdot H_2O$, while dimer B crystallized from methanol as pale yellow plates, m.p. 328° (dec.), analyzing for $C_{30}H_{18}O_{10}$. The UV absorption spectra of both A and B [λ_{max}^{EtOH} (log ε): 270 mμ (4·80), 335 mμ (4·44) (inflexion) and 268 mμ (4·61), 339 mμ (4·51) respectively] were characteristic of the known biflavonyls, exhibiting enhanced absorption at the short wavelength band in comparison to apigenin [λ_{max} (log ε): 269 mμ (4·27), 300 mμ (4·13) (inflexion), 340 mμ (4·32)] which exhibits strongest absorption at the long wavelength band. The m.ps and UV spectra of both compounds differed from those reported for amentoflavone, cupressuflavone and hinokiflavone, mixed m.ps being depressed in each case, establishing the synthetic dimers as a novel biflavonyl type.

Both dimers were readily acetylated with acetic anhydride-sodium acetate to give hexa-acetates and methylated with dimethyl sulfate-potassium carbonate to yield hexamethyl ethers, indicating that C—C coupling of the apigenin moieties was involved in each case and not C—O coupling as in hinokiflavone (IV). An examination of the 100 MHz NMR spectra of these derivatives established unequivocally the mode of coupling present in each dimer.

The NMR spectrum of dimer A hexamethyl ether (Fig. 1a) was remarkably simple, suggesting a symmetrical mode of coupling. The methoxyl groups appeared as three 6-proton singlets at 3·75, 3·83 and 3·88 ppm. In the low-field region of the spectrum a pair of two-proton doublets at 6·32 and 6·39 ppm, $J = 2\cdot0$ Hz, corresponded to the *meta*-coupled 6- and 8-protons of the A rings, while the eight B ring protons occurred as an A_2B_2 quartet at 6·67 and 7·12 ppm. Since all the aromatic protons present in

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* Yields based upon recovered apigenin (62%).

apigenin were thus accounted for, while the signal corresponding to the proton at position 3 of the pyrone ring was absent, the structure of dimer A was established as 3,3-biapigeninyl (V). This was confirmed by the spectrum of the hexa-acetate which showed the O-acetyl groups as three 6-proton singlets at 2·28, 2·33 and 2·42 ppm, the B ring protons as a pair of doublets at 6·86 and 7·16 ppm, $J = 2{\cdot}5$ Hz, and the A ring protons as a broad singlet at 6·98 ppm.

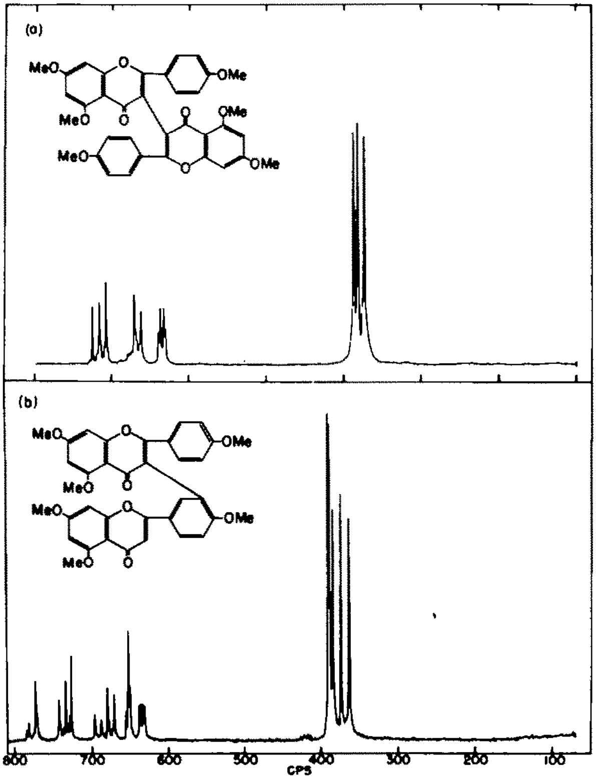
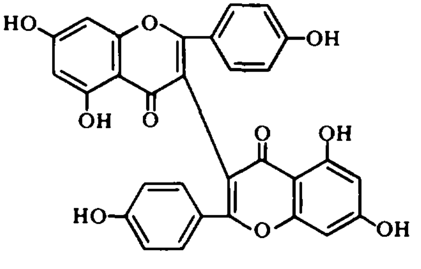


FIG. 1. 100 Mc NMR spectra, TMS as internal reference. (a) Dimer A hexamethyl ether. (b) Dimer B hexamethyl ether.

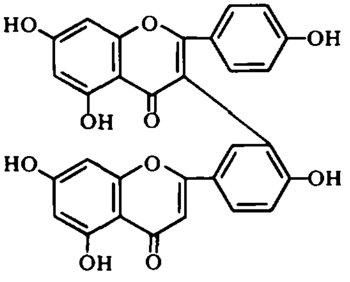
The NMR spectra of dimer B hexamethyl ether and hexa-acetate exhibited more complex patterns in the low field region, indicative of an unsymmetrical type of C—C coupling. An examination of the spectrum of the methyl ether (Fig. 1b) showed that the A rings of each moiety were not involved in the coupling, since a pair of doublets could be distinguished at 6·32 and 6·51 ppm, $J = 2{\cdot}0$ Hz, and a second pair at 6·37 and 6·54 ppm, $J = 2{\cdot}0$ Hz, accounting for the 6- and 8-protons of each ring. Superimposed upon the low-field sets of doublets was a one-proton singlet at 6·52 ppm which could only be assigned to a proton at position 3, showing that the 3-position in the other half of the dimer must be involved in the bonding. An A_2B_2 quartet at 6·75 and 7·38 ppm, accounting for four protons, confirmed the presence of one unsubstituted B ring, leaving only two alternatives for the interflavonyl linkage, namely 3—2′ or 3—3′, of which the latter appeared to be the most acceptable on a theoretical basis.

The remaining unassigned low field signals could only be interpreted as supporting the 3—3′ bond. Thus the proton at position 5′ occurred as a doublet at 6·92 ppm, whereas the 2′ proton appeared as a doublet at 7·72 ppm, superimposed upon the quartet at 7·75 and 7·83 ppm due to the 6′ proton. The 5′ proton exhibited *ortho*-coupling ($J = 8{\cdot}0$ Hz) and the 2′ proton *meta*-coupling ($J = 2{\cdot}5$ Hz) with the proton at position 6′. Since both the 2′ and 6′ proton signals occurred at lower field than that of the 5′ proton, due to the deshielding effect of the double bond in the pyrone ring upon the B ring protons within its proximity, the 3—2′ mode of linkage is excluded. In the latter case only one proton, namely that at the 6′ position, would have a signal at low field and this would be an *ortho*-coupled doublet.

The NMR spectrum of the hexa-acetate served to confirm the 3—3′ assignment for the interflavonyl bond, having a similar pattern at low field to the methyl ether but with the signal due to the proton at position 3 appearing as a singlet at 6·41 ppm, upfield from the aromatic proton signals. The acetoxy groups appeared as six singlets at 2·19, 2·25, 2·33, 2·36, 2·39 and 2·41 ppm. Dimer B must therefore have the 3,3′-biapigeninyl structure (VI).



V



VI

The mass spectrum of dimer A (Fig. 2) showed a molecular ion at mass 538 having elemental composition $C_{30}H_{18}O_{10}$ (538·08998; measured mass, 538·08963). Loss of CO from the molecular ion was suppressed to less than 0·3% relative to the molecular ion indicating substitution at the 3 position of both pyrone rings. This behavior is analogous to that observed for structurally similar isoflavone compounds.[15] The large peak for the doubly-charged molecular ion (mass 269 and 269·5) is also consistent with 3-substitution.[15]

Dimer B decomposed thermally on the direct probe of the mass spectrometer at 320° to yield phenol and a compound of mass 442 corresponding to the expected molecular weight less that of phenol. The methyl ether of B showed an intense molecular ion at mass 622 of composition $C_{36}H_{30}O_{10}$ (622·18388; measured mass, 622·17967).

It has been reported by Mahesh and Seshadri[10] that Fenton's oxidation of naringenin triacetate or 4′,7-diacetyl naringenin results in the formation of 3-hydroxy-4′,7-diacetyl naringenin, together with a product considered to be di-(4′,7-diacetoxy-5-hydroxyflavanon-3-yl) (VII). Since dehydrogenation of the latter compound, followed by hydrolysis of the acetoxy groups should lead to 3,3-biapigeninyl (V), thus confirming the structure assigned to dimer A, an attempt was made to prepare the biflavanone (VII). However, Fenton's oxidation of either naringenin triacetate or 4′,7-diacetyl

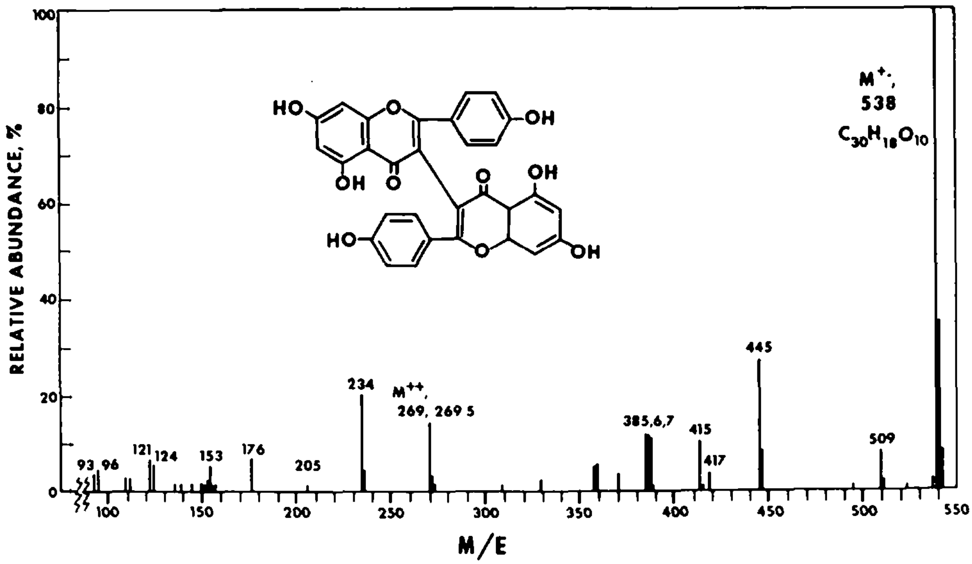
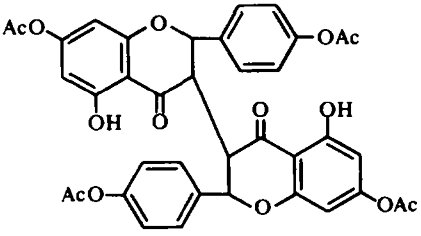


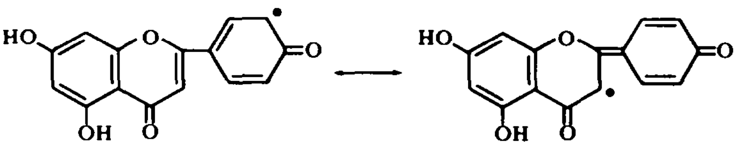
FIG. 3 Mass spectrum of Dimer A obtained by direct introduction at 280° on a CEC 21-110 double focusing mass spectrometer. Only peaks above 1·5% relative abundance are shown.

naringenin under a variety of conditions, including those reported by Mahesh and Seshadri,[10] led to quantitative recovery of starting material in every case.



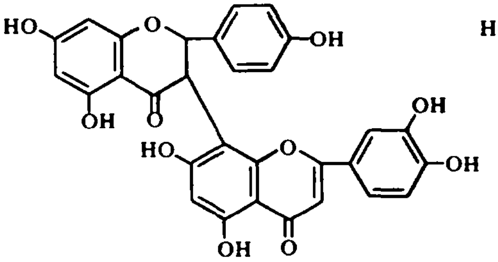
VII

The 3,3 and 3,3′ types of linkage, established for these two synthetic biflavonyls, do not correspond to the types of C—C bonding observed in those naturally occurring biflavonyls so far isolated. The synthetic compounds appear to arise by oxidative coupling of the radical (VIII), although none of the symmetrical 3′,3′-linked dimer, which might also be expected to be formed, could be isolated. All three of the above modes of coupling have been observed in the laboratory with p-hydroxy styrenes and coniferyl alcohol, which may be regarded as the basic monomers for lignan and lignin biosynthesis, respectively.[2, 3]

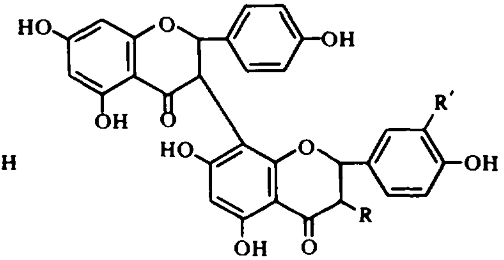


VIII

It is interesting to note that the 3,8-flavonylflavanone (IX)[11] and a series of 3,8-biflavanonyls (X)[12] have recently been isolated from *Garcinia* sp., showing that the 3 position can be involved in the interflavonyl linkages of natural biflavonyls. It has been suggested[12] that the biflavanones (X) are formed by oxidative coupling of cinnamic acid precursors, flavones or chalcones. However, the involvement of the latter in oxidative dimerization appears to conflict with the results of Dean and Podimuang,[16] who found that 2',4-dihydroxychalcones undergo intramolecular coupling on treatment with potassium ferricyanide to form 4'-hydroxyaurones in good yield.



IX



X a: R = OH, R' = H
b: R = R' = H
c: R = R' = OH

In contrast to the natural biflavonyls isolated up to the present time, the synthetic dimers did not exhibit linkage through the A ring. However, in a recent study[13] of the EPR spectra of flavonoid anion radicals produced on aerial, alkaline oxidation, the magnitude of the hyperfine coupling constants indicated that the total electron density was located on the 3-position of the pyrone ring and in the B ring, except in those flavonoids which lack an oxygen function at position 5. Radical coupling reactions would therefore not be expected to involve the A ring.

These results thus appear to indicate that the natural biflavonyls are formed not by radical coupling but rather by electrophilic attack of the radical (VIII) on a second molecule of apigenin. Such a possibility has been suggested by Baker *et al.*[14] with substitution occurring at positions 6 or 8, since these would be the most susceptible to electrophilic attack.

EXPERIMENTAL

Oxidation of apigenin with $K_3Fe(CN)_6$. Apigenin (5·0 g) was dissolved in a soln of Na_2CO_3 (20 g) in deaerated H_2O (500 ml) and a soln of $K_3Fe(CN)_6$ (12·2 g, 2 equivs) in deaerated H_2O (300 ml) added dropwise under an atmosphere of N_2 during 1 hr. The reaction mixture, which assumed a deep red–brown color, was stirred for a further 1 hr and carefully acidified with 4N·HCl soln (150 ml). The buff-colored ppt was filtered off, washed thoroughly with H_2O and air-dried, yield, 4·9 g.

TLC of apigenin oxidation product. The crude reaction product was examined by TLC on silica gel in toluene–ethyl formate-formic acid.[8] On examination under UV light three major spots could be detected with R_f values of 0·37, 0·40 and 0·55 respectively. Under identical conditions amentoflavone had R_f 0·38, hinokiflavone had R_f 0·47 and apigenin had R_f 0·55.

Chromatography of apigenin oxidation product. A portion (0·5 g) of the crude oxidation product was chromatographed on a column of Sephadex LH-20 (500 g) in MeOH, the eluate being continuously monitored by its UV absorption at 254 mμ. In this manner three fractions were collected.

Isolation of apigenin. Fraction 1 from the chromatographic separation gave a yellow solid on evaporation of the solvent which crystallized as yellow needles, m.p. 348–349° (EtOH-H_2O), undepressed on admixture with apigenin. Yield, 0·31 g (62%). Acetylation of a portion of this material with Ac_2O-NaOAc gave white needles, m.p. 183–184° (EtOH), identical in all respects with apigenin triacetate.

Isolation of 3,3-biapigeninyl (dimer A) (V). Evaporation of fraction 2 gave a buff-colored solid which crystallized as pale yellow plates, m.p. 315–317° (EtOAc-H_2O). Yield, 23 mg (5%, Based on recovered apigenin, 11%); UV, $\lambda_{\max}^{\mathrm{EtOH}}$ (log ε): 270 mμ (4·80) and 335 mμ (4·44) (inflexion). (Found: C, 64·70; H, 3·81; $C_{30}H_{18}O_{10}\cdot H_2O$ requires: C, 64·75; H, 3·61%).

3,3-Biapigeninyl hexa-acetate. Acetylation of 3,3-biapigeninyl with Ac_2O-NaOAc for 1 hr at 100° gave a white solid which crystallized as plates, m.p. 163–164° (MeOH); UV, $\lambda_{\max}^{\mathrm{EtOH}}$ (log ε); 254 mμ (4·63) and 302 mμ (4·33); NMR, ($CDCl_3$): singlets, 2·28, 2·33 and 2·42 ppm, 6 protons each (-OAc groups) doublets, 6·86 and 7·16 ppm; $J = 2·5$ c/s, 2 protons each (A ring protons); broad singlet, 6·98 ppm, 8 protons (B ring protons). (Found: C, 63·60; H, 3·92; $C_{42}H_{30}O_{16}$ requires: C, 63·78; H, 3·83%).

3,3-Biapigeninyl hexamethyl ether. Methylation of 3,3-biapigeninyl with Me_2SO_4 in Me_2CO over K_2CO_3 under reflux for 3 hr gave pale yellow plates, m.p. 186–188° (dec.) (EtOH); UV, $\lambda_{\max}^{\mathrm{EtOH}}$ (log ε): 265 mμ (4·56) and 310 mμ (4·36) (inflexion). NMR, ($CDCl_3$): singlets, 3·75, 3·83 and 3·88 ppm, 6 protons each (-OMe groups); doublets, 6·32 and 6·39 ppm, $J = 2·0$ c/s, 2 protons each (A ring protons); A_2B_2 quartet, 6·67 and 7·12 ppm, 8 protons (B ring protons). (Found: C, 69·65; H, 4·83; $C_{36}H_{30}O_{10}$ requires: C, 69·44; H, 4·86%).

Isolation of 3,3′-biapigeninyl (dimer B) (VI). Fraction 3 gave a buff-colored solid on evaporation of the solvent which crystallized as pale yellow plates, m.p. 328° (dec) (MeOH), yield: 40 mg (8Π. Based on H recovered apigenin, 21%); UV, $\lambda_{\max}^{\mathrm{EtOH}}$ (log ε): 268 mμ (4·61) and 339 mμ (4·51). (Found: C, 66·90; H, 3·31; $C_{30}H_{18}O_{10}$ requires: C, 66·92; H, 3·34%).

3,3′-Biapigeninyl hexa-acetate. Acetylation of 3,3′-biapigeninyl with Ac_2O-NaOAc for 1 hr at 100° gave a solid which crystallized as white needles, m.p. 195–197° ($CHCl_3$-MeOH). UV, $\lambda_{\max}^{\mathrm{EtOH}}$ (log ε): 255 mμ (4·52) and 302 mμ (4·45); NMR, ($CDCl_3$): singlets, 2·19, 2·25, 2·33, 2·36, 2·39 and 2·41 ppm, 3 protons each (-OAc groups); singlet, 6·41 ppm, 1 proton (proton at unsubstituted 3-position); doublets, 6·82 and 6·90 ppm, $J = 2·0$ c/s, 1 proton each (A ring 6-position protons); doublets, 7·24 and 7·35 ppm, $J = 2·0$ c/s, 1 proton each (A ring 8-position protons); A_2B_2 quartet, 7·08 and 7·46 ppm, 4 protons (unsubstituted B ring); doublet, 7·41 ppm, $J = 8·0$ c/s, 1 proton (B ring 5′ proton); doublet, 7·46 ppm, $J = 2·0$ c/s, 1 proton (B ring 2′ proton); quartet, 7·79 and 7·87 ppm, $J = 2·0$ and 8·0 c/s, 1 proton (B ring 6′ proton). (Found: C, 63·70; H, 3·83; $C_{42}H_{30}O_{16}$ requires: C, 63·78; H, 3·83%).

3,3′-Biapigeninyl hexamethyl ether. Methylation of 3,3′-biapigeninyl with Me_2SO_4 in Me_2CO over K_2CO_3 under reflux for 3 hr gave pale yellow needles, m.p. 264–265° (MeOH); UV, $\lambda_{\max}^{\mathrm{EtOH}}$ (log ε): 264 mμ (4·67) and 322 mμ (4·60); NMR, ($CDCl_3$): singlets, 3·65, 3·75, 3·86, 3·90, 3·91 and 3·92 ppm, 3 protons each (-OMe groups); doublets, 6·32 and 6·37 ppm, $J = 2·0$ c/s, 1 proton each (A ring 6-position protons); doublets, 6·51 and 6·54 ppm, $J = 2·0$ c/s, 1 proton each (A ring 8-position protons); singlet, 6·52 ppm, 1 proton (proton at unsubstituted 3-position); A_2B_2 quartet, 6·75 and 7·38 ppm, 4 protons (unsubstituted B ring); doublet, 6·92 ppm, $J = 8·0$ c/s, 1 proton (B ring 5′ proton); doublet, 7·72 ppm, $J = 2·5$ c/s, 1 proton (B ring 2′ proton); quartet, 7·75 and 7·83 ppm, $J = 2·5$ and 8·0 c/s, 1 proton (B ring 6′ proton). (Found: C, 69·40; H, 5·00; $C_{36}H_{30}O_{10}$ requires: C, 69·44; H, 4·86%).

Attempted oxidation of naringenin triacetate and 4′,7-diacetylnaringenin by Fenton's reagent. Attempted oxidation of either naringenin triacetate or 4′,7-diacetylnaringenin in 2N H_2SO_4 solution with H_2O_2 and aqueous $FeSO_4$ solution at 0° according to the method of Mahesh and Seshadri[10] led to quantitative recovery of starting material. Identical results were obtained when the reaction was carried out at different temperatures and with alternative sequences of addition of oxidizing agents.

Acknowledgements—The authors are indebted to L. M. White and Miss G. Secor for elemental analyses, Mrs N. Bennett for measurement of NMR spectra and Mrs G. Stark for technical assistance.

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