Extractives from Guttiferae. Part 34.[1] Kolaflavanone, a New Biflavanone from the Nuts of *Garcinia kola* Heckel. Applications of ^{13}C Nuclear Magnetic Resonance in Elucidation of the Structures of Flavonoids

By **Philip J. Cotterill** and **Feodor Scheinmann,*** Department of Chemistry and Applied Chemistry, University of Salford, Salford M5 4WT
Ian A. Stenhouse, P.C.M.U., Harwell, Didcot, Oxfordshire OX11 0RE

The nuts of *Garcinia kola* Heckel contain 3,8-linked biflavanones and triterpenes. A new metabolite, kolaflavanone, has been characterised as the II-4'-methyl ether of GB-2, as part of a ^{13}C n.m.r. study of flavonoids.

THE false kola nut, *Garcinia kola* Heckel, of Nigeria and Ghana is chewed habitually by the local population as a synergist with the true kola nut.[2] In local medicine the masticated nut pulp is used for its antiseptic action

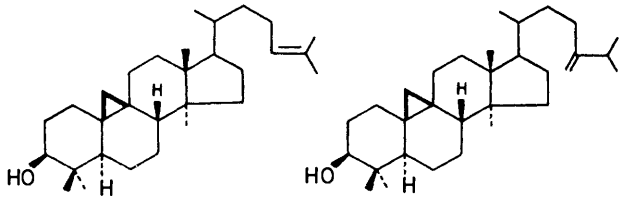
[1] Part 33, M. S. b.Hj. Idris, A. Jefferson, and F. Scheinmann, *J.C.S. Perkin I*, 1977, 2158.

[2] R. T. Aplin, J. H. C. Blasdale, T. G. Halsall, and G. M. Hornby, *J. Chem. Soc.* (*C*), 1967, 246.

in the treatment of cuts and for the prevention of sore throats and as an antidote, effective against North-West African arrow poisons.[3]

Extraction of the dried, milled nuts with chloroform gave a gum which was separated by column chromatography into two groups of compounds. The first was a mixture of triterpenes and further investigations by g.l.c.–mass spectrometric methods confirmed the presence of cycloartenol (1) and 24-methylenecycloartenol (2). These results were consistent with those reported by Aplin *et al.*[2]

The second group of compounds was shown by t.l.c. to be a closely related series of four highly polar metabolites, which were identified as I-3,II-8 linked flavanone–flavanone dimers (3)—(6). The occurrence of I-3,II-8 biflavanones (GB biflavanones [4]) is restricted



(1) (2)

to members of the *Garcinia* species [4] (Fam. Guttiferae; Sub-fam. Clusoideae) and its near neighbour *Pentaphalangium* (*P. solomonse* Warb.).[5] The isolation of a new biflavanone of the GB series,[4] which we have named kolaflavanone (3), is now reported.

An ethyl acetate extract of the defatted nuts gave a solid containing exclusively the four biflavanones. Some prior separation of this mixture was achieved by extraction with sodium borate,[6] and preparative t.l.c. gave the known metabolites, GB-1a (4), GB-1 (5), GB-2 (6), and the new biflavanone, kolaflavanone. Mass spectrometric, n.m.r., and t.l.c. comparisons with authentic samples confirmed the identity of GB-1a, GB-1, and GB-2 (4)—(6).

Kolaflavanone (3) gave the characteristic i.r. and u.v. spectra of the GB biflavanones [4] and the molecular ion at *m/e* 588 showed a fragmentation pattern similar to those of GB-1 and GB-2 (Scheme 1). A hydroxy-group was located at position II-3 by loss of 18 m.u. from the molecular ion. A difference of 30 m.u. between GB-1 and kolaflavanone suggested that kolaflavanone had an extra methoxy-group and its position must be restricted to ring I-B or II-B since the elements of a hydroxymethoxybenzyl fragment gave a peak at *m/e* 137 ($C_8H_9O_2$) (7). Further, fragment ions at *m/e* 270 ($C_{15}H_{10}O_5$) (8) and 296 ($C_{16}H_8O_6$) (9), which are also given by GB-1 and GB-2, shows that the aromatic ring I-B has the same substituents, and therefore the methoxy-group must be located in ring II-B (Scheme 1).

[3] Professor J. Ene, Benin University, Nigeria, personal communication.

[4] H. D. Locksley, *Fortschr. Chem. org. Naturstoffe*, 1973, **30**, 207.

[5] P. J. Owen and F. Scheinmann *J.C.S. Perkin I*, 1974, 1018.

The n.m.r. spectrum of kolaflavanone shows good correlation with that recorded for GB-2, confirming the hydroxy-substituent in position II-3, the di-oxygenation pattern of ring II-B, and the presence of one methoxy-group. A more detailed study of the complex aromatic signals of kolaflavanone was made by using a 220 MHz instrument. Thus the AA'BB' quartet from the proton of ring I-B appears at τ 2.97 and 3.35 (d, J 8.5 Hz) and the protons of ring II-B give a complex set of signals at τ 3.17 (d, J 8 Hz, H-5'), 3.19 (d, J 2 Hz, H-2'), and 3.29 (q, J 2 and 8 Hz, H-6'). The remaining aromatic protons resonate as doublets at τ 4.18 (J 2 Hz) and 4.24 (J 2 Hz) for H-6 and H-8, respectively, of ring I-A while H-6 of ring I-A gives a singlet at τ 4.16. With the methoxy-singlet at τ 6.27 the remaining signals are due to the ring C protons at τ 4.50 (d, J 12 Hz) and 5.51 (d, J 12 Hz) for I-C and τ 5.10 (d, J 12 Hz) and 5.96 (d, J 12 Hz) for II-C, respectively.

Alkaline degradation studies (Scheme 2) were used to assist in the location of the methoxy-group at position II-3' or II-4'. Shortage of pure kolaflavanone, however, meant that identification of the degradation products had to be limited to t.l.c. analysis only. Comparison of the degradation products with authentic samples clearly indicated the absence of 4-hydroxy-3-methoxybenzoic acid, but since 3-hydroxy-4-methoxybenzoic acid and 4-hydroxybenzoic acid (derived from ring I-B) could not be separated by t.l.c., the presence of 3-hydroxy-4-methoxybenzoic acid was implied but not confirmed. ^{13}C N.m.r. spectroscopy was used to confirm the 3-hydroxy-4-methoxy-substitution pattern of ring II-B in kolaflavanone.

The ^{13}C n.m.r. spectra of some simple flavonoids were interpreted by using a set of empirical results, for substituted aromatic systems, reported by Elvidge [7] (Table 1). Thus for naringenin (10) the proton-noise-decoupled spectrum shows 13 lines which account for the

TABLE 1

^{13}C N.m.r. substituent effects for monosubstituted benzenes [a] (in p.p.m. relative to benzene at 129 p.p.m. from Me_4Si)

Substituent	1-C	*o*-C	*m*-C	*p*-C
COMe	9.3	0.2	0.2	4.2
CH_2OH	12.3	−1.4	−1.4	−1.4
OPh	29.2	−9.4	1.6	−5.1
OMe	30.2	−14.7	0.9	−8.1
OH	27.6	−11.8	2.6	−6.1
Me	9.1	0.3	0.3	2.8

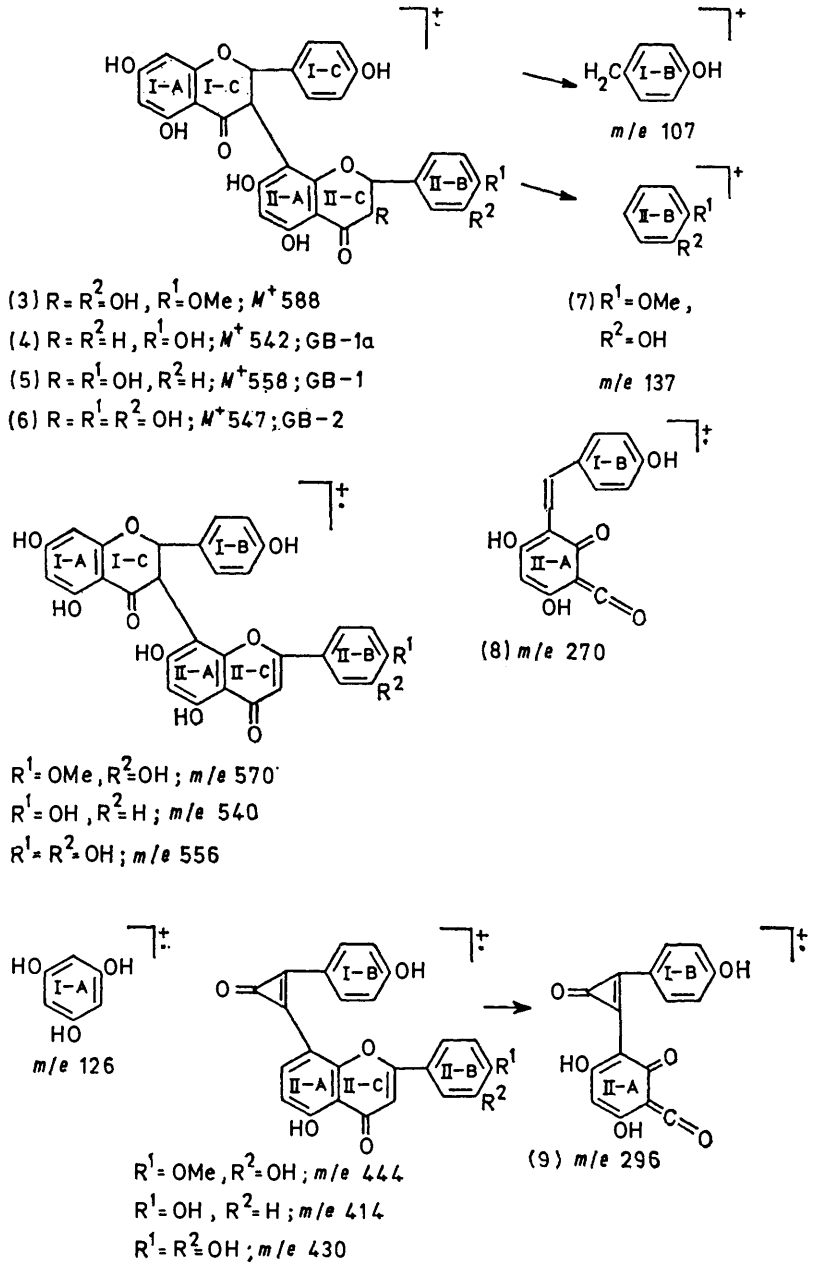
[a] Data from J. A. Elvidge in 'An Introduction to Spectroscopic Methods for the Identification of Organic Compounds,' ed. F. Scheinmann, Pergamon, Oxford, 1974, vol. 2, p. 229.

15 carbon atoms of naringenin (10) since C-2' and C-3' (see numbering system used in Scheme 3) of ring B are chemically and magnetically equivalent to C-6' and C-5',

[6] B. Jackson, H. D. Locksley, F. Scheinmann, and W. A. Wolstenholme, *Tetrahedron Letters*, 1967, 787; *J. Chem. Soc.* (*C*), 1971, 3791.

[7] J. A. Elvidge in ' An Introduction to Spectroscopic Methods for the Identification of Organic Compounds,' vol. 2, ed. F. Scheinmann, Pergamon, Oxford, 1974, 222.

respectively. The off-resonance-decoupled spectrum of naringenin identified C-3 of ring C by showing a signal as a triplet as 43.35 p.p.m. The signal due to the carbonyl carbon atom C-4 was also readily assigned, at 197.05 p.p.m., since it resonates at lowest field. The remaining signals were assigned using Table 1, and from by assuming that the effect of multiple substituents is additive, the chemical shift for each aromatic carbon atom was calculated. Further approximations were also introduced since some substituents in naringenin are not included in the Table. Thus ring C of naringenin (10) was approximated to a methylene alcohol substituent



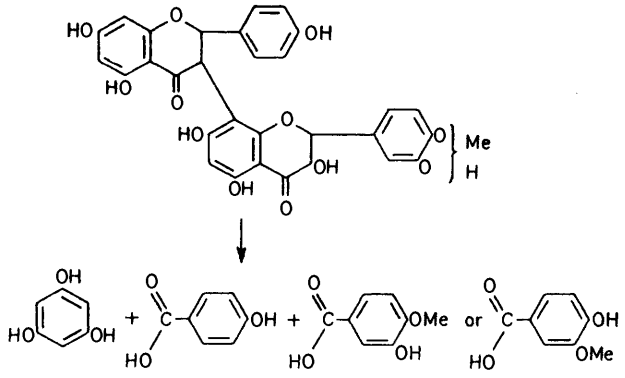
SCHEME 1

the coupling observed in the off-resonance-decoupled spectrum.

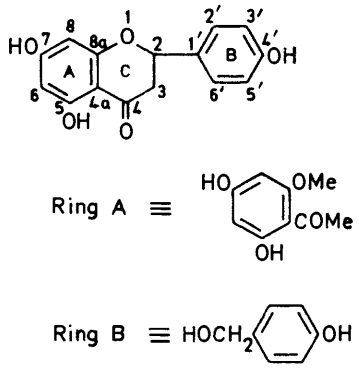
The aromatic assignments of rings A and B are derived from the chemical shift of the carbon atoms of benzene, which resonate at *ca.* 129 p.p.m., and calculation of the influence of substituents in the ring (Table 1). Thus, for ring B and to a methoxy and a methyl ketone group for ring A (Scheme 3).

Comparison of the calculated and the observed chemical shifts for naringenin showed good agreement and most of the signals were easily assigned (Table 2). As expected from the calculations there is some ambi-

guity in ring A with the signals assigned to C-6 and -8 and also to C-5, -7, and -8a since the assignments within

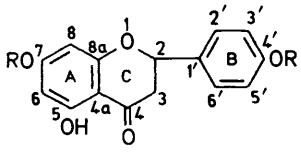


SCHEME 2

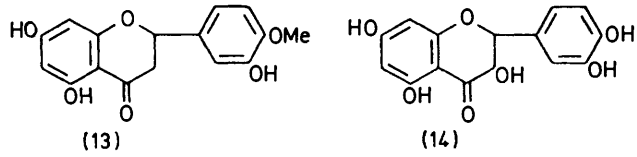


SCHEME 3 Aromatic substitution patterns used to assign ^{13}C n.m.r. chemical shifts of naringenin

these two groups of signals can be interchanged. The assignments given in Table 2, chosen to agree with the (160.3 p.p.m.) and observed (165.1 p.p.m.) chemical shift occurs at C-5 because the empirical calculations do not take account of intramolecular hydrogen bonding which occurs between the hydroxy-group at C-5 and the carbonyl at C-4. This effect is thus seen as an additional downfield shift in the signal assigned to C-5. The effect of intramolecular hydrogen-bonding on the chemical shift of the carbonyl carbon atom in xanthones [1,11] and flavones has been discussed previously.[9,10]



(10) R = H
(11) R = Me
(12) R = H and $\Delta^{2,3}$



(13) (14)

The effect of intramolecular hydrogen bonding was also observed in the comparison of the ^{13}C n.m.r. spectra of *o*- and *p*-hydroxyacetophenones: the hydroxy-carbon atom shows a significant additional downfield shift in the *ortho*-isomer only. Thus a comparison of calculated and experimental values (Table 3) for the *o*-isomer showed good correlation for C-3, -4, and -6 but where chelation modifies the environments of the carbon atoms, *i.e.* at C-1, -2, and -5 (*para* to hydroxy-group) deviations are

TABLE 2

^{13}C N.m.r. spectral data for monoflavanoids (splitting in off-resonance-decoupled spectrum) in p.p.m. from Me_4Si *

Compound		Ring A						Ring B						Ring C			Others
		5	6	7	8	8A	4A	1'	2'	3'	4'	5'	6'	2	3	4	MeO
Naringenin	Found	165.1 *a* (s)	96.8 (d)	167.2 *a* (s)	95.8 (d)	164.2 *a* (s)	103.1 (s)	130.7 (s)	128.9 (br,s)	116.1 (br,s)	158.5 (s)	116.1 (br,s)	128.9 (br,s)	79.8 (d)	43.4 (t)	197.1 (s)	
	Calc.	160.3	97.5	164.3	96.6	164.6	105.7	135.2	130.2	115.8	155.2	115.8	130.2				
Naringenin-4,7-Dimethyl ether	Found	164.2 (s)	95.1 (d)	167.9 (s)	94.2 (d)	162.9 (s)	103.1 (s)	130.5 (s)	127.7 (m)	114.3 (m)	160.1 (m)	114.3 (m)	127.7 (m)	79.0 (d)	43.2 (t)	196.0 (s)	55.6 (q) +55.3 (q)
	Calc.	158.6	94.6	166.9	93.7	162.9	103.2	133.2	128.5	112.9	157.8	112.9	128.5				55.6
Apigenin	Found	157.2 *b* (s)	99.8 (d)	164.0 *b* (s)	93.9 (d)	163.7 *b* (s)	103.7 (s)	121.2 (s)	128.2 (br,s)	115.9 (br,d)	161.1 *b* (s)	115.9 (br,d)	128.2 (br,s)	161.4 *b* (s)	102.8 (d)	181.6 (s)	
	Calc.	160.3	97.5	164.3	96.6	164.6	105.7	135.2	130.2	115.8	155.2	115.8	130.2	162.2	126.5		
Hesperetin	Found	163.5 *c* (s)	95.9 (d)	166.6 *c* (s)	95.0 (d)	162.7 *c* (s)	101.8 (s)	131.2 (s)	112.0 (m)	146.5 *d* (s)	147.9 *d* (s)	114.1 (d)	117.6 (m)	78.2 (d)	42.1 (t)	196.0 (s)	55.6 (q)
	Calc.	160.3	97.5	164.3	96.6	164.6	105.7	135.8	116.7	140.5	146.0	115.5	122.4				55.6
Taxifolin	Found	163.3 *e* (s)	96.1 (d)	166.8 *e* (s)	95.0 (d)	162.5 *e* (s)	100.5 (s)	128.1 (s)	115.4 (m)	144.9 *f* (s)	145.7 *f* (s)	115.4 (d)	119.5 (m)	83.1 (d)	71.6 (d)	197.5 (s)	
	Calc.	160.3	97.5	164.3	96.6	164.6	105.7	137.8	118.4	143.4	143.4	118.4	124.1				

* Solvent $(CD_3)_2SO$.

a-f Values may also be interchanged with assignments bearing the same superscript for acceptable agreement with calculated data. However, the chosen assignments are are in closest agreement with reported ^{13}C n.m.r. data for flavanoids.

empirical calculations, are supported by the assignments for ring A carbons based on ^{13}C–^{1}H spin–spin coupling to the chelated hydroxy-proton [8] and other data for flavanoids.[9,10]

The most significant discrepancy between calculated found. On the other hand the *para*-isomer showed good agreement for all carbon atoms except C-1. To check these results, *p*-methoxyacetophenone was also examined (Table 3) and the values were found to be in

[8] F. W. Wehrli, *J.C.S. Chem. Comm.*, 1975, 663.
[9] K. R. Markham and B. Ternai, *Tetrahedron*, 1976, **32**, 2607, 565.
[10] H. Wagner, V. M. Chari, and J. Sonnenbichler, *Tetrahedron Letters*, 1976, 1799.
[11] J. S. E. Holker, R. D. Lapper, and T. J. Simpson, *J.C.S. Perkin I*, 1974, 2135.

excellent agreement. Thus the 'chelation effect' appears to produce a downfield shift on the hydroxy-carbon atom of about 5.5 p.p.m.

A similar series of calculations and approximations for naringenin 4,7-dimethyl ether (11) (Table 2) gave a set of figures also in good agreement with the experimental results as well as those found for naringenin. The chemical shifts in apigenin (12) proved much more changed (and did in fact increase significantly) was the higher field of the two signals between 140 and 150 p.p.m. Again this is what would be expected from the calculated shifts but the shift difference is not as large as predicted.

These measurements thus gave the assignments shown in Table 3. Some conclusions can now be made about the spectra of these two compounds which can be used for assigning the structure of ring II-B in kolaflavanone

TABLE 3

^{13}C N.m.r. data for acetophenone and benzyl alcohol derivatives, in p.p.m. from Me_4Si* (splittings in off-resonance-decoupled spectrum)

								Others			
		1	2	3	4	5	6	C=O	Me	OMe	CH_2OH
Compound	Found	119.7	162.3	118.8 [a]	136.3	118.2 [a]	130.7	204.4	26.3		
2-Hydroxyacetophenone		(s)	(s)	(d)	(d)	(d)	(d)	(s)	(q)		
	Cal.	126.5	156.8	117.4	135.8	123.1	131.8				
	Found	129.0	130.8	115.4	162.2	115.4	130.9	196.8	26.1		
4-Hydroxyacetophenone		(s)	(d)	(d)	(s)	(d)	(d)	(s)	(q)		
	Calc.	132.2	131.8	117.4	161.0	117.4	131.8				
	Found	130.6	130.6	113.8	163.6	113.8	130.6	196.2	26.1	55.4	
4-Methoxyacetophenone		(s)	(d)	(d)	(s)	(d)	(d)	(s)	(q)	(q)	
	Calc.	130.2	130.1	114.5	163.6	114.5	130.1			55.6	
	Found	133.5	115.0	147.3 [b]	145.3 [b]	111.1	119.1			55.5	63.0
4-Hydroxy-3-methoxybenzyl		(s)	(m)	(s)	(s)	(d)	(m)			(q)	(t)
alcohol	Calc.	136.1	115.5	146.0	140.5	116.7	122.1			55.6	
	Found	135.2	112.0	146.3 [c]	146.5 [c]	114.3	117.3			55.7	62.8
3-Hydroxy-4-methoxybenzyl		(s)	(m)	(s)	(s)	(d)	(m)			(q)	(t)
alcohol	Calc.	135.8	116.7	140.5	146.0	115.5	122.4			55.6	

* Solvent $(CD_3)_2SO$. [a–c] Values may be interchanged with assignments bearing the same superscript for acceptable agreement with calculated data.

difficult to rationalise; some of the calculated values gave a poor fit with the observed signals (Table 2). However, the approximations used to obtain the calculated values do not allow for the increased conjugation in ring C which substantially influences the chemical shifts of ring B. The effect of intramolecular hydrogen bonding is not so significant as for flavanones and the chemical shift for C-5 of ring A is much nearer the calculated value (Table 2).

The model compounds 4-hydroxy-3-methoxy- and 3-hydroxy-4-methoxy-benzyl alcohol which correspond to the two possible isomeric forms of ring II-B for kolaflavanone were next investigated (Table 3). The signals could not be assigned unambiguously by comparing the proton-decoupled chemical shifts with the calculated values. However, by using the following techniques, the unambiguous assignment given in Table 3 was obtained.

In each case an off-resonance spectrum showed one aromatic signal split into a clean doublet (indicated d in Table 3); two others showed basic doublet structure with residual longer range coupling (indicated as m) superimposed. This long-range coupling must be residual $^3J_{CH}$, which can only occur for C-2 and -6, and the clear doublet is thus due to C-5. This was confirmed by long-range coupling patterns in the ^1H-coupled spectra.

The assignment of C-3 and -4 was achieved by measuring relaxation times (T_1) before and after D_2O exchange. This should give rise to an increase in T_1 for the aromatic carbon atom with the hydroxy-group attached but no change for any other T_1. For each compound it was found that the only signal whose T_1 and flavones in general. The protonated carbon atom next to the OH group occurs at highest field (*ca.* 111—112 p.p.m.). In an off-resonance spectrum C-5 appears as a clean doublet; the other signals from C-2 and -6 show multiplet structure. The carbon atom with the hydroxy-group attached resonates at higher field than that with the methoxy-group.

To demonstrate the applicability of the above conclusions and in the hope of finding a model compound more closely related to kolaflavanone, the ^{13}C n.m.r. spectrum of hesperetin (13) was measured (Table 2). The same approximations as used for naringenin gave calculated chemical shifts in good agreement with the experimental values (Table 2) for ring A.

The three diagnostic signals for the substitution pattern of ring B in the region 110—120 p.p.m. for C-2′, -5′, and -6′ have chemical shifts and splitting patterns in the off-resonance decoupled spectrum [a multiplet (112.0 p.p.m.), a doublet (114.1 p.p.m.), and a multiplet (117.6 p.p.m.)] similar to those previously observed for 3-hydroxy-4-methoxybenzyl alcohol.

The ^{13}C n.m.r. spectrum of kolaflavanone was not so definitive owing to the small amount of material available and the large number of scans required to produce the spectrum. However, by comparison with the reference data obtained from the monoflavanones, the benzyl alcohols, and GB-1a and GB-2 it was possible to assign the various carbon resonances to rings A, B, and C of the two flavanone components in kolaflavanone (Table 4). The region from 110 to 120 p.p.m. contains three groups of signals attributed to C-3′ and -5′ of ring I-B, which as expected coincides with the resonance due to C-5′ of ring

II-B at 114.8 p.p.m. The signal at next highest field (111.8 p.p.m.) is attributed to C-2′ of ring II-B but the signal in the region of 118 p.p.m. due to C-6′ of ring II-B was only weak, and although seen in the spectrum its intensity was not above the threshold value to be recorded by the computer. The off-resonance-decoupled spectrum of kolaflavanone shows that the signals at *ca.* 112 and 118 p.p.m. have been considerably broadened to represent the resonances at C-2′ and -6′ of ring II-B as multiplets whereas the signal at 114.8 p.p.m. shows only slight broadening due to overlap of two doublets. These results indicate that kolaflavanone has a 3′-hydroxy-4′-methoxy-substitution pattern in ring II-B by comparison with the spectra of hesperetin (13) and 3-hydroxy-4-methoxybenzyl alcohol. In contrast, the isomeric structure with a 3′-methoxy-4′-hydroxy-substitution pattern in ring II-B should show the highest field carbon signal due to C–H adjacent to the hydroxy-group at C-5′ as a doublet in the off-resonance-decoupled spectrum as observed in 4-hydroxy-3-methoxybenzyl alcohol.

Thus by analysis of the C–H coupling in ring II-C in the ^{13}C spectrum of kolaflavanone (3), the presence of a hesperetin (13) moiety is indicated. The combined spectral and degradative evidence therefore supports the structural assignment of kolaflavanone (3) as having a naringenin unit (10) linked at C-3 to ring A of the hesperetin (13) moiety.

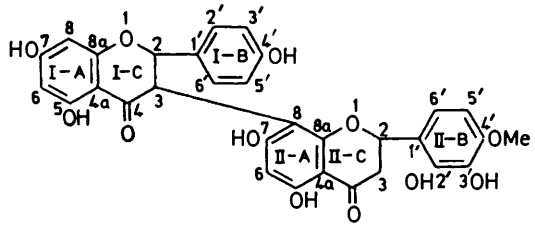
The assignment of the other signals in the ^{13}C spectrum of kolaflavanone (3) follows from comparison of the ^{13}C spectra of the two monomeric flavanone components and from calculated chemical shifts. Although there are some discrepancies between the calculated and found chemical shifts (Table 4), it is possible to group the

TABLE 4

^{13}C N.m.r. chemical shifts (p.p.m. from Me_4Si) of kolaflavanone * and comparisons with the calculated values for the aromatic protons †

Signal	Assignments (calculated values)
47.3	I-C-3
55.6	OMe
82.7	I-C, II-C-2
101.3, 101.1, 96.1, 95.0	I-A-6 I-A-8 I-A-4a II-A-6 II-A-8 II-A-4a (97.5) (96.6) (105.5) (97.8) (105.7) (106.0)
111.8	II-B-2′ (116.7)
114.8	I-B-3′ and 5′ II-B-5′
129.8, 128.8, 127.7, 126.7	I-B-1′ 2′ and 6′ II-B-1′ (135.2) (130.2) (135.8)
146.2	II-B-3′ II-B-4′ (140.5) (146.0)
157.7	I-B-4′ (152.2)
166.4, 164.6, 163.7, 162.7, 162.1	I-A-5 I-A-7 I-A-8a II-A-5 II-A-7 II-A-8a (160.3)(164.3)(164.6)(163.1)(164.6)(164.9)
197.4, 196.5	I-C-4 II-C-4

* The numbering used is as follows:



† Calculations follow from those used for naringenin and hesperitin in Table 2. The incremental value for the substituent at II-A-8 is taken as equivalent to that of a methyl group in Table 1. Assignments are supported by the coupling observed in the off-resonance-decoupled spectrum.

TABLE 5

^{13}C N.m.r. chemical shifts (p.p.m. from Me_4Si) of GB-1a * and comparisons with the calculated values for the aromatic protons †

Signal	Assignment (calculated values)
47.4	I-C-3 or II-C-3
78.3, 81.3	I-C-2 II-C-2
95.0, 96.1	I-A-6 (97.5) I-A-8 (96.6) II-A-6 (9.78)
101.3	I-A-4a (105.7) II-A-8 (105.7) II-A-4a (106.0)
114.6, 115.1	I-B-3′ and 5′ (115.8) II-B-3′ and 5′ (115.8)
126.6, 128.9	I-B-2′ and 6′ (130.2) II-B-2′ and 6′ (130.2)
128.0	I-B-1′ (135.2) II-B-1′ (135.2)
157.5	I-B-4′ (152.2) II-B-4′ (152.2)
162.2, 162.7, 163.7, 164.8, 166.3	I-A-5 (160.2) I-A-7 (164.3) I-A-8a (164.6) II-A-5 (163.1) II-A-7 (164.6) II-A-8a (164.9)
195.9, 196.7	I-C-4 II-C-4

* The numbering used is as in Table 4.

† Calculations follow from those used for naringenin in Table 2. The incremental value for the substituent at II-A-8 is taken as equivalent to that of a methyl group in Table 1. Assignments are supported by the coupling observed in the off-resonance-decoupled spectrum.

TABLE 6

^{13}C N.m.r. chemical shifts (p.p.m. from Me_4Si) of GB-2 * and comparison with the calculated values for the aromatic protons †

Signal	Assignment (calculated values)
45.2	I-C-3
70.0	II-C-2 or II-C-3
79.6	I-C-2
80.8	II-C-2 or II-C-3
93.0, 94.0	I-A-6 (97.5) I-A-8 (96.6) II-A-6 (97.8)
99.2	IA-4a (105.7) II-A-6 (105.7) II-A-4a (106.0)
112.8	I-B-3′ and 5′ (115.8) II-B-2′ and 5′ (118.4)
125.9, 126.1	I-B-1′ II-B-1′ (135.2) (137.8)
126.9	I-B-2′ and 6′ (130.2) II-B-6′ (124.4)
142.8, 143.6	II-B-3′ and 4′ (143.4)
155.7	I-B-4′ (155.2)
160.0, 160.7, 161.7	I-A-5 (160.2) I-A-7 (164.3) II-A-5 (163.1) II-A-7 (164.6)
162.7, 164.4	I-A-8a (164.6) II-A-8a (164.9)
194.5, 195.4	I-C-4 II-C-4

* The numbering used is as in Table 4.

† Calculations follow from those used for naringenin and taxifolin in Table 2. The incremental value for the substituent at II-A-8 is taken as equivalent to that of a methyl group in Table 1. Assignments are supported by the coupling observed in the off-resonance decoupled spectrum.

signals which arise from carbon atoms in similar environments in the two flavanone units.

As expected, the ^{13}C spectrum of GB-1a (4) resembles the spectrum of naringenin (10) and again it is possible to assign groups of signals to carbon atoms in similar environments by comparison with calculated values (Table 5).

For the analysis of GB-2, which consists of a naringenin unit (10) linked to taxifolin (14), the ^{13}C spectrum of taxifolin was examined: for most of the carbon atoms the calculated and found chemical shifts are in good agreement (Table 2). The most marked anomalies arise from the chelation effect in ring A and from C-1′ in ring B. The ^{13}C spectrum of GB-2 in many features resembles the composite spectra of naringenin (10) and taxifolin (14) and a comparison of the calculated and found chemical shifts for GB-2 (Table 6) also shows discrepancies due to C-1′ in rings I-B and II-B.

In calculating the chemical shifts of the biflavanones one further approximation was required in determining the chemical shifts of ring II-A. It was assumed that the naringenin substituent at II-A-8 has a substituent effect on chemical shift equivalent to that of a methyl group (Table 1). After these ^{13}C n.m.r. studies were complete,[12] spectral data of monoflavanoids were reported [8-10,13,14] which are complementary to this study.

EXPERIMENTAL

U.v. spectra (solutions in methanol) were recorded with a Unicam SP 800 spectrophotometer and i.r. spectra (Nujol mulls) with a Perkin-Elmer 257 grating spectrophotometer. Mass spectra were obtained from an A.E.I. MS 12 spectrometer operating at 240 °C and 70 eV. ^1H N.m.r. spectra were recorded [solutions in $(CD_3)_2SO$ at 100 °C with hexamethyldisiloxane as high temperature lock] on a Varian HA 100 instrument and for kolaflavanone, at room temperature, on a Varian HR 220 instrument. ^{13}C N.m.r. spectra were measured on a Bruker HX 90 instrument at Harwell and a CFT 20 spectrometer at Salford. Silica gel G (nach Stahl; Merck) was used for analytical t.l.c., silica gel HF 254 (Merck) for preparative t.l.c., and silica gel MFC (Hopkin and Williams) for column chromatography.

The nuts of *Garcinia kola* Heckel (Guttiferae) from Kumasi, Benin, and Ibadan (Nigeria and Ghana) were air dried until the brown outer skin could be removed and the nuts were hard and brittle. They were then milled through a 1.5 mm screen using an Apex milling machine. The milled nuts (from Kumasi, 1 180 g; Benin, 556 g; Ibadan, 1 072 g) were continuously extracted separately with hot chloroform (8 l) for 8 h. Concentration of the solutions gave black viscous oils (Kumasi, 56.8 g; Benin, 25.9 g; Ibadan, 56.6 g), and comparative t.l.c. on the three oils indicated that each had the same distribution of metabolites. A portion of the Kumasi extract (6 g) was separated by column chromatography (300 g of silica gel) by increasing the polarity of the eluent from hexane through to methanol. The concentrated eluates were combined into a series of crude fractions showing similar t.l.c. characteristics.

Many fractions consisted of oily mixtures and were not investigated further. However, one of these fractions slowly precipitated a white mixture which was readily recrystallised from acetone to give an amorphous white powder (0.475 g), m.p. 88—92°. The steroidal composition was determined by g.l.c.–mass spectroscopic analysis, and the mixture was shown with the aid of authentic samples to be a 50 : 50 mixture of cycloartenol and 24-methylenecycloartenol, $\nu_{max.}$ (mixture) 3 440b, 1 348, 1 295, 1 110, 1 060, 1 035, 1 015, 1 000, and 895 cm^{-1}, M^+ 440 for 24-methylenecycloartenol and 426 for cycloartenol.

The defatted nuts were further extracted with ethyl acetate (4 l); removal of the solvent under reduced pressure gave crispy brown solids containing the four biflavanones (Kumasi, 55.2 g; Benin, 33.2 g; Ibadan, 50.2 g). The biflavanone mixture (10 g) in ethyl acetate (100 ml) was given a preliminary separation by extraction with sodium tetraborate solution (0.1M; 100 ml). The extraction was repeated twice more to give three aqueous solutions which were acidified and extracted with ethyl acetate (100 ml). The dried ($MgSO_4$) ethyl acetate solutions were concentrated to small volume under reduced pressure and filtered onto a large excess of light petroleum (b.p. 60—80 °C), to give pale brown amorphous solids: 1st borate extract, 3.25 g; 2nd, 1.71 g; 3rd, 1.48 g; borate-insoluble, 2.35 g.

Preparative t.l.c. (80 : 20 chloroform–methanol) separated the components of the mixture to give GB-1a (concentrated in the borate-insoluble portion), GB-1 (concentrated in the second and third borate extracts), GB-2 (concentrated in the first borate extract), and kolaflavanone (concentrated in the second, third, and borate-insoluble extracts). The separated biflavanones were isolated as brown oils which when dissolved in ethyl acetate and filtered onto a large excess of light petroleum (b.p. 60—80 °C) gave off-white, amorphous powders. GB-1a had R_F 0.55 (20% methanol in chloroform), $\nu_{max.}$ 3 350br, 1 640, 1 520, 1 168, 1 092, and 838 cm^{-1}, τ 3.05 (4 H, t, J 8 Hz), 3.47 (4 H, t, J 8 Hz), 4.29 (3 H, s), 4.58 (1 H, d, J 12 Hz), 4.71 and 4.82 (1 H, q, J 5 and 11 Hz), 5.58 (1 H, d, J 12 Hz), and 7.44 (2 H, m).

GB-1 had R_F 0.50 (20% methanol in chloroform); $\lambda_{max.}$ 293 and 328 nm; $\nu_{max.}$ 3 360br, 1 638, 1 520, 1 270, 1 170, 1 091, and 838 cm^{-1}; τ −2.13 (1 H, s), −1.70 (1 H, s), 3.02 (4 H, t, J 8 Hz), 3.42 (4 H, t, J 8 Hz), 4.28 (3 H, d), 4.62 (1 H, d, J 12 Hz), 5.14 (1 H, d, J 12 Hz), 5.62 (1 H, d, J 12 Hz), and 6.02 (1 H, d, J 12 Hz).

GB-2 had R_F 0.425 (20% methanol in chloroform); $\nu_{max.}$ 3 340br, 1 634, 1 515, 1 280, 1 179, 1 090, and 837 cm^{-1}, τ 3.08 (2 H, d, J 8 Hz), 3.46 (5 H, m), 4.30 (3 H, d), 4.59 (1 H, d, J 12 Hz), 5.27 (1 H, d, J 11 Hz), 5.61 (1 H, d, J 12 Hz), and 6.07 (1 H, d, J 11 Hz).

Kolaflavanone had R_F 0.525 (20% methanol in chloroform), $\lambda_{max.}$ 292 (ε 32 000) and 329 nm (6 900); $\nu_{max.}$ 3 360br, 1 640, 1 520, 1 280, 1 170, 1 091, and 840 cm^{-1}; τ −1.99 (1 H, s), −1.59 (1 H, s), 3.05 and 340 (7 H, m), 4.30 (3 H, d), 4.60 (1 H, d, J 12 Hz), 5.20 (1 H, d, J 12 Hz), 5.62 (1 H, d, J 12 Hz), 6.08 (1 H, d, J 12 Hz), and 6.36 (3 H, s) [Found: M^+, 588; m/e 444.083 7 ($C_{25}H_{16}O_8$ requires 444.084 4); m/e, 270.053 5 ($C_{15}H_{10}O_5$ requires 270.052 8); m/e 137.060 2 ($C_8H_9O_2$ requires 137.060 3)].

Methylation of the biflavanone mixture gave an inseparable complex mixture. However, mass spectrometry clearly showed the presence of the biflavanone methyl

[12] P. J. Cotterill, Ph.D. Thesis, University of Salford, 1975.
[13] A. Pelter, R. S. Ward, and T. I. Gray, *J.C.S. Perkin I*, 1976, 2475.
[14] C. A. Kingsbury and J. H. Looker, *J. Org. Chem.*, 1975, **40**, 1120; P. Joseph-Nathan, J. Mares, Ma. C. Hernandez, and J. N. Shoolery, *J. Magnetic Resonance*, 1974, **16**, 447; V. M. Chari, M. Jordan, H. Wagner, and P. W. Thies, *Phytochemistry*, 1977, **16**, 1110.

ethers. Thus with dimethyl sulphate and potassium carbonate in acetone, GB-1a and GB-1 hexamethyl ethers showed M^+ 626 and 642, respectively, and GB-2 and kolaflavanone formed heptamethyl ethers, M^+ 672. With methyl iodide and silver(I) oxide in dimethylformamide, GB-1a, GB-2, and kolaflavanone formed their permethyl ethers, M^+ 626, 656, and 686, respectively.

Alkaline Degradations of Kolaflavanone.—(a) *Fusion.* Kolaflavanone (50 mg) was added to molten sodium hydroxide (0.5 g) and the solution stirred for 0.5 h. The cooled mixture was diluted with water (25 ml) and acidified (2M-HCl). The solution was extracted with chloroform (2 × 10 ml), which was then back-extracted with aqueous sodium hydrogen carbonate (1M; 2 × 10 ml). The acidified (2M-HCl) aqueous extract was finally extracted with chloroform (2 × 10 ml). T.l.c. of the two organic solutions showed mainly phloroglucinol in the hydrogen carbonate-insoluble layer and in the chloroform extract of the acidified hydrogen carbonate solution a spot corresponding to either 4-hydroxybenzoic acid or 3-hydroxy-4-methoxybenzoic acid or a mixture of the two was seen.

(b) *Alkaline peroxide.* Kolaflavanone (50 mg) was dissolved in a solution containing hydrogen peroxide (100 vol; 5 ml) at 18 °C and sodium hydroxide (0.25 g). The solution was warmed slowly until effervescence had ceased and finally heated to 100 °C for 15 min. The diluted, acidified (2M-HCl) solution was treated as above; t.l.c. gave a similar comparable set of results.

We thank the S.R.C. for a grant (to P. J. C.), and Professor J. Ene (Benin), Professor G. O. Phillips, Professor F. Kufuor (Kumasi), and the Tropical Products Institute, London, for help with supply of samples.

[7/1238 *Received*, 13*th July*, 1977]