Erythrina Studies. Part 2.[1] Structures of Three Novel Prenylated Antibacterial Flavanones, Sigmoidins A—C, from *Erythrina sigmoidea* Hua

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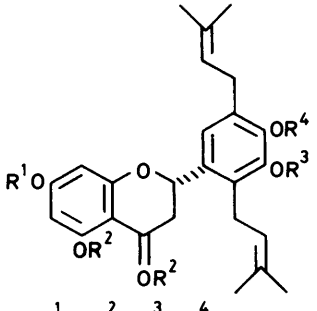
Three new prenylflavanones, (**1**), (**6**), and (**10**), have been isolated from the chloroform extract of *Erythrina sigmoidea* Hua (Fabaceae) bark and characterised. Two of the three compounds exhibit noteworthy antibiotic activity against Gram-positive bacteria. Their structures were elucidated from spectral data and chemical correlations.

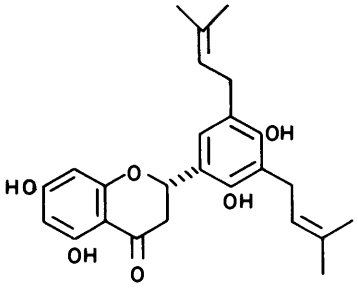
The genus *Erythrina* is well known for its alkaloids.[2] Its neutral components have, on the other hand, attracted little attention. In the only major work on the neutral compounds of this taxon, Nakanishi and co-workers[3] isolated from *Erythrina abyssinica* several flavanoids which displayed significant biological activity. As a result of our continuing studies[4] on Cameroonian medicinal plants, the neutral chloroform extract of the widely used folk medicinal plant, *E. sigmoidea*,[5] was found to show antibacterial activity at 1 000 p.p.m. A preliminary communication[1] described the structural determination of the antibacterial flavanones, sigmoidin A (**1**) and sigmoidin B (**6**), isolated from the extract. In the present paper we present a full account of the isolation and structural elucidation of the two compounds (**1**) and (**6**) as well as the companion flavanone, sigmoidin C (**10**).

The chloroform extract of the dried powdered bark of *E. sigmoidea* Hua collected at Foumban in the Western Province of Cameroon was subjected to silica gel column chromatography with solvents of increasing polarity to give stigmasterol and the three flavanones.

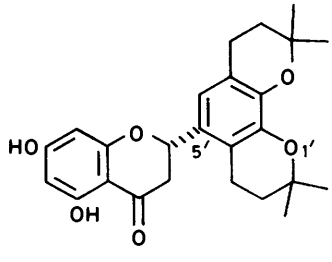
Structure of Sigmoidin A (**1**).—Sigmoidin A was isolated as colourless needles, m.p. 181—182 °C, $[\alpha]_D$ −82° in methanol, and its molecular formula was determined as $C_{25}H_{28}O_6$ from elemental, analytical, and mass spectral data. Colour tests with iron(III) chloride (green) and magnesium–conc. hydrochloric acid (pink), together with the u.v. spectral data (see Experimental section), indicated that sigmoidin A was a flavanone bearing at least two hydroxy groups.[6] Further indication of the flavanone skeleton came from the i.r. spectrum of sigmoidin A which exhibited strong absorptions at $\nu_{max.}$ 3 500 (free OH), 3 300—3 100 (chelated OH), and 1 640 (chelated flavanone C=O). The trimethyl ether of sigmoidin A (M^+, 466) from diazomethane methylation gave a green colour with iron(III)



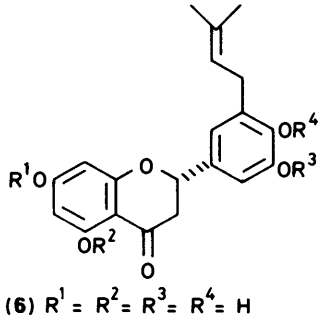
(**1**) $R^1 = R^2 = R^3 = R^4 = H$
(**2**) $R^1 = R^3 = R^4 = Me, R^2 = H$
(**3**) $R^1 = R^2 = R^3 = R^4 = Ac$



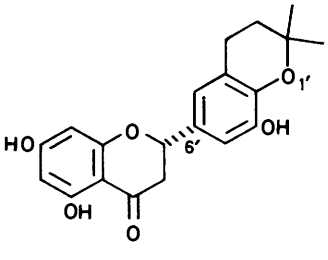
(**4**)



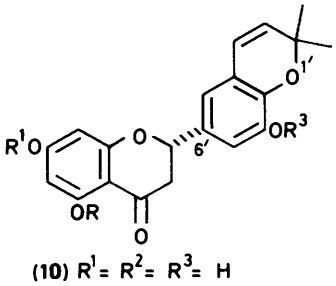
(**5**)



(**6**) $R^1 = R^2 = R^3 = R^4 = H$
(**7**) $R^1 = R^3 = R^4 = Me, R^2 = H$
(**8**) $R^1 = R^2 = R^3 = R^4 = Ac$



(**9**)



(**10**) $R^1 = R^2 = R^3 = H$
(**11**) $R^1 = R^2 = R^3 = Ac$
(**12**) $R^1 = R^3 = Me, R^2 = H$

chloride, thus confirming the presence of a chelated hydroxy group, while acetylation with acetic anhydride–pyridine yielded a tetra-acetate which did not respond to the iron(III) chloride test. Thus sigmoidin A contains four hydroxy groups. The bathochromic shifts in the u.v. spectrum of sigmoidin A induced by sodium methoxide, sodium acetate, and aluminium chloride are consistent with its formulation as a flavanone hydroxylated at C-5 and C-7.[6] The ^{1}H n.m.r. spectrum [$(CD_3)_2SO$] of sigmoidin A confirmed its 5,7-dihydroxylated nature and showed resonances for the characteristic flavanone 3-H_2 and 2-H protons at δ 2.80 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.16 (1 H, dd, *J* 17 and 4 Hz, 3-H), and 5.33 (1 H, m, 2-H), and four D_2O-exchangeable signals for four hydroxy groups [δ_H 7.85, 8.05, 10.40, and 12.01 (all singlets)].

Further resonances for three aromatic protons were observed at δ_H 5.84 (2 H, s) and 6.70 (1 H, s). The former was assigned to 6- and 8-H in accord with chemical-shift data recorded for these two protons[8] while the latter signal obviously arose from ring B.

The presence of two 3-methylbut-2-enyl (prenyl) groups in sigmoidin A was suggested by ^{1}H n.m.r. signals at δ_H 1.67 (12 H, br s), 3.24 (4 H, d, *J* 7.5 Hz), and 5.28 (2 H, crude t), and mass fragments at *m/z* 369 ($M^+ - C_4H_7$), 314 ($M^+ - 2 \times C_4H_7$), 356 ($M^+ - C_5H_8$), and 288 ($M^+ - 2 \times C_5H_8$). It thus followed that sigmoidin A is a 5,7-dihydroxyflavanone bearing four substituents: two prenyl and two hydroxy groups in ring B whose relative positions had to be determined. On biogenetic grounds,[7] it was assumed that there would be hydroxylation at C-4′. Furthermore, in sigmoidin A tetra-acetate the 6- and 8-H singlet in the ^{1}H n.m.r. spectrum at δ_H 5.84 was split into two symmetric doublets (*J* 2 Hz) and shifted to δ_H 6.65 and 6.80 while the ring-B proton singlet remained almost unchanged at δ_H 6.68. The absence of any downfield shift for this proton on acetylation indicated that it was not *ortho* to a hydroxy group.[8,9] Hence two possible structures (**1**) and (**4**) could be assigned to sigmoidin A. Brief * and mild treatment of sigmoidin A with 98% formic acid furnished a single bis-chromane derivative (**5**) which lacked prenyl absorptions in its ^{1}H n.m.r. spectrum. This result ruled out the possibility of compound (**4**) which must give more than two chromane derivatives in this reaction. Sigmoidin A could therefore be formulated as (**1**).

Sigmoidin A (**1**), to our knowledge, is the first flavanone to have as many as four substituent groups in ring B.

Structure of Sigmoidin B (**6**).—Sigmoidin B, m.p. 217—218 °C, $[\alpha]_D^{28}$ −54°, was isolated from chloroform–methanol as colourless granules, and was determined to have the molecular formula $C_{20}H_{20}O_6$ by mass spectrometry and elemental analysis. Preliminary colour tests [green with iron(III) chloride and pink with magnesium–conc. hydrochloric acid[10]] showed that sigmoidin B was also a hydroxylated flavanone. The i.r. and u.v. spectra of sigmoidin B (see Experimental section) further indicated that this compound had the same 5,7-dihydroxylation pattern as did sigmoidin A.[7] The ^{1}H n.m.r. spectrum of sigmoidin B was well resolved and showed, besides the characteristic 3-H_2 and 2-H resonances, signals for one chelated hydroxy group at δ_H 12.02 and one 3-methylbut-2-enyl group. Resonances for four aromatic protons were also observed at δ_H 5.86 (2 H, s), 6.61 (1 H, d, *J* 2 Hz), and 6.75 (1 H, d, *J* 2 Hz). The former were assigned to 6- and 8-H while the other two obviously arose from ring B. In agreement with the above spectral data sigmoidin B, on diazomethane methylation, afforded a trimethyl ether and, on acetylation, a tetra-acetate. Sigmoidin B therefore differed from sigmoidin A (**1**) only in having one fewer prenyl group in ring B. The establishment of its structure thus resolved itself into the problem of determining the positions of the prenyl and the two hydroxy groups. One of the hydroxy groups was assigned to C-4′ on biogenetic considerations.[9] Brief treatment of sigmoidin B with 98% formic acid gave a single dimethyldihydropyran derivative whose spectral data were consistent with structure (**9**), thus indicating that the prenyl group was flanked by a single hydroxy group. The existence of two *meta*-coupled ring-B protons (^{1}H n.m.r. spectrum *vide supra*) coupled with the above evidence uniquely defined the B-ring substitution pattern as 3′,4′-dihydroxy-5′-(3-methylbut-2-enyl). Hence the novel structure 3′,4′,5,7-tetrahydroxy-5′-(3-methylbut-2-enyl)flavanone (**6**) was assigned to sigmoidin B.

Structure of Sigmoidin C (**10**).—Sigmoidin C was obtained as colourless granules from chloroform–methanol, m.p. 222 °C. The molecular formula $C_{20}H_{18}O_6$ was derived from mass spectral (M^+, 354) and elemental analysis. The u.v. spectral pattern and shifts in various u.v. shift reagents (see Experimental section) and characteristic absorption at 1 640 cm^{-1} in the i.r. spectrum of sigmoidin C indicated that it also was a 5,7-dihydroxylated flavanone. The u.v. spectrum particularly resembled that of sigmoidin B, therefore suggesting the same 3′,4′,5,5′,7-substitution pattern for sigmoidin C. The formation of a triacetate indicated the presence of three hydroxy groups, and the formation of only a dimethyl ether with an excess of diazomethane supported the (assumed) chelated nature of one of these hydroxy groups. The ^{1}H n.m.r. spectrum of sigmoidin C was very similar to that of sigmoidin B (**6**). The only difference was the presence of signals from a 2,2-dimethylchromene ring [δ_H 1.40 (6 H, s), 5.70 (1 H, d, *J* 10 Hz), and 6.35 (1 H, d, *J* 10 Hz)] in sigmoidin C instead of the signals assigned to a 3-methylbut-2-enyl group in compound (**6**). On the basis of these results, structure (**10**) was proposed for sigmoidin C. Confirmation of this structural assignment was readily obtained from the following transformation. Formic acid-cyclisation of sigmoidin B (**6**) afforded the 2,2-dimethyldihydropyran (**9**) whose suspension in dry benzene was readily dehydrogenated[11] to give compound (**10**), identical in all respects with the natural sample.

The absolute stereochemistry of sigmoidin A (**1**), sigmoidin B (**6**), and sigmoidin C (**10**) is assumed to be 2*S* as illustrated since all natural (−)-flavanones have been shown to have the *S*-chirality at C-2.[12]

All the flavanones isolated in this study were tested for their antimicrobial activity by standard tube-dilution techniques using both representative Gram-positive and -negative organisms and fungi as references.[13] Weak *in vitro* activity was confirmed against *Staphylococcus aureus*, *Bacillus subtilis*, and *Trichophyton mentagrophytes* for sigmoidin A (**1**) and against *S. aureus* for sigmoidin B (**6**).

Experimental

General Procedures.—All m.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H N.m.r. spectra were recorded on Perkin-Elmer R 12 and R 32 spectrometers in $CDCl_3$ and $(CD_3)_2SO$ solutions with Me_4Si as internal standard. Mass spectra were obtained with an LKB 9000S instrument, and specific rotations in methanol on an AA-100 electronic polarimeter. I.r. spectra were run as KBr discs on a Perkin-Elmer 727 B, and u.v. spectra on a Beckmann 25 spectrophotometer. Silica gel GF_{254} (Merck) and silica gel 60 (70—230 mesh ASTM) (Merck) were used for t.l.c. and column chromatography, respectively. Microanalytical results were obtained from the University of Glasgow.

* Prolonged treatment was avoided to prevent erratic rearrangements of the prenyl group of the type recently reported by Wu *et al.* (T. S. Wu, H. Furukawa, C. S. Kuo, and K. S. Hsu, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1681) which could otherwise cast doubt on the assignment of the position of these groups.

Methylations were performed with an excess of diazomethane in methanol–diethyl ether during 8 h at 0—5 °C, while acetylations were in acetic anhydride–dry pyridine at room temperature. 'Work-up' refers to dilution of the reaction mixture with water, extraction with ether, and washing of the ether layer with water. Organic solutions were dried over sodium sulphate and evaporations were performed under reduced pressure at 60 °C in a rotatory evaporator.

Extraction and Separation.—The air-dried pulverised stem bark of *Erythrina sigmoidea* Hua (5 kg) collected at Foumban in the Western Province of Cameroon during February 1982 was successively extracted with n-hexane, chloroform, and methanol. Only the chloroform extract (350 g, 7%) was examined in this investigation. Part of this extract (100 g) was column chromatographed over silica gel (1.2 kg) packed in n-hexane. Gradient elution was effected with hexane–diethyl ether mixtures, chloroform and chloroform–methanol mixtures. A total of 220 fractions of *ca.* 250 ml per fraction were collected and mixed on the basis of t.l.c. and ^{1}H n.m.r data. The pure compounds were obtained from the combined fractions either by direct crystallisation or after further purification by column chromatography or preparative t.l.c. (p.l.c.).

Stigmasterol.—Crystallisation of the combined fractions 42—48 eluted with hexane–diethyl ether (9:1) gave stigmasterol as needles (200 mg, 1.4×10^{-2}%), m.p. 168—170 °C (from ethanol). It was identical (i.r. and mixed m.p.) with an authentic sample.

Sigmoidin A (**1**).—The combined fractions 75—85 eluted with hexane–diethyl ether (3:2) were concentrated to give a brown gum. Treatment with chloroform afforded a white solid (1.5 g) which was crystallised from chloroform–methanol to give *sigmoidin A* (**1**) (0.105%) as needles, m.p. 181—182 °C; $[\alpha]_D^{28}$ −82° (*c* 2.0 in methanol) (Found: M^+, 424; C, 70.53; H, 6.7. $C_{25}H_{28}O_6$ requires *M*, 424; C, 70.74; H, 6.41%); $\lambda_{max.}$(MeOH) 288 nm ($\varepsilon_{max.}$ 12 000); $\lambda_{max.}$(MeOH + NaOMe) 323 nm ($\varepsilon_{max.}$ 20 900); $\lambda_{max.}$(MeOH + NaOAc) 325 nm ($\varepsilon_{max.}$ 18 800); $\lambda_{max.}$(MeOH + $AlCl_3$) 309 nm ($\varepsilon_{max.}$ 15 400); $\lambda_{max.}$(MeOH + $AlCl_3$ + HCl) 309 nm ($\varepsilon_{max.}$ 15 400); $\nu_{max.}$ 3 500 (OH), 3 300—3 100 (chelated OH), 1 640 (flavanone C=O), 1 600, 1 500 (aromatic C=C), 1 440, 1 380, 1 290, 1 180, 1 160, 1 080, 1 060, 1 010, 980, 860, 830, 815, and 720 cm^{-1}; δ_H [90 MHz; $(CD_3)_2SO$] 1.67 (12 H, br s), 2.80 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.16 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.24 (4 H, d, *J* 7 Hz), 5.28 (2 H, ill defined overlapping triplet), 5.33 (1 H, m, 2-H), 5.84 (2 H, s, 6- and 8-H), 6.70 (1 H, s, 6′-H), and four signals which disappeared on deuteriation, respectively at δ_H 7.85 (1 H, s), 8.05 (1 H, s), 10.40 (1 H, s), and 12.01 (1 H, s); *m/z* 424 (M^+, 100%).

Methylation of Sigmoidin A (**1**).—Methylation of sigmoidin A (**1**) (200 mg) with an excess of diazomethane in methanol-diethyl ether, followed by the usual work-up, afforded a viscous oil (225 mg) containing one major product (t.l.c.). Chromatography on silica gel with n-hexane–methylene dichloride (95:5) as eluant yielded *sigmoidin A trimethyl ether* (**2**) (160 mg), m.p. 96—98 °C (from n-hexane–diethyl ether) (Found: M^+, 466; C, 71.9; H, 7.5. $C_{28}H_{34}O_6$ requires *M*, 466; C, 72.08; H, 7.35%); $\lambda_{max.}$(EtOH) 228sh (11 700) and 288 nm (8 400); $\nu_{max.}$ 3 400, 1 635, 1 565, 1 490, 1 430, 1 370, 1 295, 1 180, 1 150, 1 085, 1 045, 998, 970, 880, 860, and 820 cm^{-1}; δ_H ($CDCl_3$; 90 MHz) 1.66 (6 H, s, Me_2C=), 1.70 (6 H, s, Me_2C=), 2.80 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.10 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.32 (4 H, d, *J* 7.5 Hz, 2 × CH_2CH=), 3.77 (9 H, s, 3 × OMe), 5.10 (2 H, m, 2 × CH_2CH=CMe_2), 5.42 (1 H, m, 2-H), 6.02 (2 H, br s, 6- and 8-H), 7.10 (1 H, s, 6′-H), and 12.05 (1 H, s, exchanged with D_2O, 5-OH).

Acetylation of Sigmoidin A (**1**).—Acetylation of sigmoidin A (**1**) (100 mg) with acetic anhydride (2 ml) in pyridine (4 ml), followed by the customary work-up, afforded *sigmoidin A tetraacetate* (**3**) as an oil (85 mg) which resisted all attempts at crystallisation (Found: M^+, 592; C, 68.9; H, 6.5. $C_{33}H_{36}O_{10}$ requires *M*, 592; C, 69.20; H, 6.34%); $\lambda_{max.}$(MeOH) 260 nm (11 000); $\nu_{max.}$ 1 770s (ester C=O), 1 690s (ring C=O), 1 610s, 1 460s, 1 420s, 1 190 (ester C–O), and 1 020 cm^{-1}; δ_H ($CDCl_3$; 60 MHz) 1.65 (12 H, br s, 2 × Me_2C=), 2.25 (9 H, s, 3 × Ac), 2.35 (3 H, s, 5-OAc), 2.80 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.30 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.24 (4 H, d, *J* 7.5 Hz, 2 × CH_2CH=), 5.05 (2 H, m, 2 × CH_2CH=), 5.32 (1 H, m, 2-H), 6.5 (1 H, d, *J* 2.5 Hz, 8-H), 6.71 (1 H, d, *J* 2.5 Hz, 6-H), and 7.10 (1 H, s, 6′-H); *m/z* 592 (M^+, 8%), 549 (47), 507 (36), 494 (28), 452 (25), 297 (50), 271 (37), 257 (58), 256 (70), 255 (73), 237 (95), 195 (58), 153 (61), and 43 (100).

Cyclisation of Sigmoidin A (**1**).—A solution of sigmoidin A (**1**) (150 mg) in formic acid (30 ml) was refluxed at water-bath temperature for 25 min. The solution was poured into cold water and extracted with chloroform. The extract was washed successively with aqueous sodium hydrogen carbonate and water, dried, and evaporated to dryness. Crystallisation of the residue from chloroform yielded the *tetrahydrobenzodipyran derivative* (**5**) (130 mg, 87%), m.p. 264—266 °C (Found: C, 70.7; H, 6.5. $C_{25}H_{28}O_6$ requires C, 70.74; H, 6.41%); $\lambda_{max.}$(MeOH) 229 (8 100) and 289 nm (7 100); $\nu_{max.}$ 3 595 (OH), 3 190 (chelated OH), 1 640 (flavanone C=O), 1 600, 1 500 (arom. C=C), 1 450, 1 290, and 820 cm^{-1}; δ_H [90 MHz; $CDCl_3$ + $(CD_3)_2$SO] 1.54 (12 H, s, 4 × Me), 1.78 (4 H, t, *J* 7 Hz, 2 × CH_2), 2.85 (4 H, t, *J* 7 Hz, 2 × CH_2), 2.87 (1 H, m, overlapping with the methylene signal, 3-H), 3.18 (1 H, dd, *J* 16.5 and 4 Hz, 3-H), 5.4 (1 H, m, 2-H), 6.03 (2 H, s, 6- and 8-H), and 6.77 (1 H, s, 5′-H).

Sigmoidin B (**6**).—Fractions 92—100 eluted by hexane–diethyl ether (1:1) gave, on concentration, a white solid (2 g, 0.14%) which was crystallised from methanol–chloroform to yield *sigmoidin B* (**6**) as granules, m.p. 217—218 °C; $[\alpha]_D^{28}$ −54° (*c* 3.0 in methanol) (Found: C, 67.2; H, 5.8. $C_{20}H_{20}O_6$ requires C, 67.40; H, 5.66%); $\lambda_{max.}$(MeOH) 288 nm (12 900); $\lambda_{max.}$(MeOH + NaOMe) 325 nm (21 800); $\lambda_{max.}$(MeOH + NaOAc) 323 nm (19 600); $\lambda_{max.}$(MeOH + $AlCl_3$) 309 nm (16 000); and $\lambda_{max.}$(MeOH + $AlCl_3$ + HCl) 309 nm (16 000); $\nu_{max.}$ 3 600 (OH), 3 450—3 150 (chelated OH), 1 635 (flavanone C=O), 1 600, 1 500 (aromatic C=C), 1 470, 1 440, 1 300, 1 150, 1 080, 1 040, 1 000, 950, and 840 cm^{-1}; δ_H [60 MHz; $(CD_3)_2SO$] 1.63 (6 H, s, Me_2C=), 2.84 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.20 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.28 (2 H, d, *J* 7 Hz, CH_2CH=), 5.22 (1 H, t, *J* 7 Hz, CH_2CH=), 5.40 (1 H, m, 2-H), 5.86 (2 H, s, 6- and 8-H), 6.61 and 6.75 (1 H, each d, *J* 2 Hz, 2′- and 6′-H), and 12.06 (1 H, s, exchangeable with D_2O, 4-OH); *m/z* 356 (M^+, 70%), 339 (13), 300 (8), 204 (20), 191 (93), 153 (100), 148 (45), 123 (18), 91 (30), 69 (45), 55 (30), and 41 (68).

Methylation of Sigmoidin B (**6**).—Methylation of sigmoidin B (**6**) (300 mg) with an excess of diazomethane in methanol–diethyl ether as described for sigmoidin A (**1**) gave an oil (280 mg). Chromatography on silica gel with n-hexane–chloroform (4:1) afforded *sigmoidin B trimethyl ether* (**7**) (260 mg), m.p. 105—107 °C (Found: C, 69.3; H, 6.7. $C_{23}H_{26}O_6$ requires C, 69.33; H, 6.58%); $\lambda_{max.}$ (MeOH) 287 nm (10 800); $\nu_{max.}$ 3 450—3 300 (bonded OH), 1 645 (flavanone C=O), 1 680, 1 500, 1 440, 1 370, 1 300, 1 280, 1 200, 1 160, 1 090, and 820 cm^{-1}; δ_H (60 MHz; CCl_4) 1.68 (6 H, br s, Me_2C=), 2.71 (1 H, dd, *J* 16.5 and 4 Hz, 3-H), 2.80 (1 H, dd, *J* 16 Hz and 4 Hz, 3-H), 3.30 (2 H, d, *J* 7 Hz, CH_2CH=), 3.75 (6 H, s, 2 × OMe), 3.90 (3 H, s, 7-OMe), 5.20 (2 H, m, 2-H and CH_2CH=), 5.94 (2 H, s, 6- and 8-H), 6.73 (2 H, q, *J* 2 Hz, 2′- and 6′-H), and 12.35 (1 H, s, disappeared on deuteriation, 5-OH); *m/z* (75 eV) 398 (M^+, 15%), 384 (100), 380

(19), 340 (12), 325 (39), 205 (36), 167 (27), 163 (13), 67 (12), and 45 (13).

Acetylation of Sigmoidin B (**6**).—Acetylation of sigmoidin B (**6**) (200 mg) as above yielded sigmoidin B tetra-acetate (**8**) as an oil (190 mg), which gave a negative response to the $FeCl_3$ test; $\lambda_{max.}$ (MeOH) 287 nm (11 000); $\nu_{max.}$ 1 765 (acetate C=O), 1 690 (flavanone C=O), 1 610 (aromatic C=C), 1 420, 1 360, 1 180 (acetate C–O), 1 120, 1 060, 1 000, and 880 cm^{-1}; δ_H (60 MHz; $CDCl_3$) 1.65 (3 H, s, Me), 1.72 (3 H, s, Me), 2.25 (9 H, s, 3 × OAc), 2.30 (3 H, s, Ac), 2.80 (1 H, dd, *J* 16.5 and 4 Hz, 3-H), 3.10 (1 H, dd, *J* 16.5 and 4 Hz, 3-H), 3.22 (2 H, d, *J* 7.5 Hz, CH_2CH=), 5.02—5.60 (2 H, m, 2-H and CH_2CH=), 6.50 (1 H, d, *J* 2 Hz, 6-H), 6.75 (1 H, d, *J* 2 Hz, 8-H), and 7.10 (2 H, s, 2′- and 6′-H); *m/z* 524 (M^+, 29%), 498 (17), 483 (27), 482 (63), 481 (35), 464 (18), 456 (12), 441 (27), 440 (95), 439 (100), 423 (18), 398 (67), 397 (50), and 84 (17).

Cyclisation of Sigmoidin B (**6**) *with Formic Acid.*—Compound (**6**) (200 mg) was heated at 90—100 °C for 25 min with formic acid (85%; 20 ml) and the mixture was then left at room temperature for 2 h. Water was added and the solution was extracted with chloroform. The extract was washed in turn with aqueous sodium hydrogen carbonate and water, dried, and concentrated. The residue showed a single spot on t.l.c.; crystallisation from methanol–chloroform furnished the *chromano derivative* (**9**) (170 mg), m.p. >280 °C (Found: C, 67.1; H, 5.8. $C_{20}H_{20}O_6$ requires C, 67.40; H, 5.66%); $\lambda_{max.}$ (MeOH) 288 nm (12 000); $\nu_{max.}$ 3 450 (free OH), 3 160 (chelated OH), 1 635 (flavanone C=O), 1 595, 1 500, 1 460, 1 365, 1 320, 1 260, 1 200, 1 080, 1 055, 1 000, 940, 920, 840, and 810 cm^{-1}; δ_H (60 MHz; $CDCl_3$) 1.30 (6 H, br s, Me_2C=), 1.80 (2 H, t, *J* 7 Hz, $ArCH_2CH_2$), 2.74 (2 H, t, *J* 7 Hz, $ArCH_2CH_2$), 2.90—3.40 (2 H, m, 3-H_2), 5.25 (1 H, m, 2-H), 5.92 (2 H, br s, 6- and 8-H), 6.70 (1 H, d, *J* 2 Hz, 5′- or 7′-H), and 6.8 (1 H, d, *J* 2 Hz, 7′- or 5′-H).

Sigmoidin C (**10**).—This was obtained as cream coloured granular crystals, m.p. 222 °C (from chloroform–methanol) (Found: C, 67.7; H, 4.95. $C_{20}H_{18}O_6$ requires C, 67.79; H, 5.12%); $[\alpha]_D^{28}$ −8° (*c* 1.0 in chloroform); $\lambda_{max.}$(EtOH) 285 nm (28 000); $\nu_{max.}$ 3 250 (OH), 1 640 (flavanone C=O), 1 600, 1 595 (aromatic C=C), 1 490, 1 460, 1 380, 1 360, 1 300, 1 250, 1 180, 1 160, 1 140, 1 080, 1 040, 940, 880, 860, and 840 cm^{-1}; δ_H [60 MHz; $(CD_3)_2SO$] 1.40 (6 H, s, Me_2C–O), 2.82 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.22 (1 H, dd, *J* 17 and 4 Hz, 3-H), 5.35 (1 H, dd, *J* 11 and 4 Hz, 2-H), 5.70 (1 H, d, *J* 10 Hz, chromene H), 5.95 (2 H, s, 6- and 8-H), 6.35 (1 H, d, *J* 10 Hz, chromene H), 6.70 (1 H, *J* 2 Hz, 5′- or 7′-H), 6.90 (1 H, d, *J* 2 Hz, 7′- or 5′-H), 8.3—8.8 (1 H, m, disappeared on deuteriation, OH), and 12.13 (1 H, s, disappeared on deuteriation, 5-OH). The signal of the 7-OH group could not be observed; *m/z* 354 (M^+, 80%), 339 (100), 187 (90), 153 (90).

Acetylation of Sigmoidin C (**10**).—Acetylation of sigmoidin C (**10**) (200 mg) with Ac_2O (5 ml) and pyridine (10 ml), followed by work-up and crystallisation from methanol–diethyl ether, afforded sigmoidin C triacetate (**11**) (180 mg) as white needles, m.p. 145—146 °C; $\lambda_{max.}$(EtOH) 284 nm (9 000); $\nu_{max.}$ 1 770 (acetate C=O), 1 690 (ring C=O), 1 620, 1 580, 1 490 (aromatic C=C), 1 440, 1 370, 1 260, 1 200 (acetate C–O), 1 120, 1 060, and 900 cm^{-1}; δ_H [60 MHz; $(CD_3)_2CO$] 1.38 (6 H, s, Me_2C), 2.23 (6 H, s, 2 × Ac), 2.50 (3 H, s, Ac), 2.80 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.20 (1 H, dd, *J* 17 and 4 Hz, 3-H), 5.50 (1 H, dd, *J* 11 and 4 Hz, 2-H), 5.78 (1 H, d, *J* 10 Hz, chromene H), 6.42 (1 H, d, *J* 10 Hz, chromene H), 6.55 (1 H, d, *J* 2 Hz, 8-or 6-H), 6.75 (1 H, d, *J* 2 Hz, 6- or 8-H), and 7.10 (2 H, s, 5′- and 7′-H); *m/z* 480 (M^+, 25%), 465 (37), 438 (41), 423 (70), 381 (48), 354 (4), 353 (8), 256 (36), 149 (90), 97 (40), 95 (34), 83 (60), 81 (85), 73 (100), and 71 (73).

Methylation of Sigmoidin C (**10**).—Treatment of sigmoidin C (**10**) (200 mg) with an excess of diazomethane in diethyl ether–methanol gave the *sigmoidin C dimethyl ether* (**12**) as cream coloured needles (192 mg), m.p. 120—121 °C (Found: C, 69.3; H, 6.1. $C_{22}H_{22}O_6$ requires C, 69.10; H, 5.80%); $\lambda_{max.}$(EtOH) 286 nm (17 200); $\lambda_{max.}$ 3 450 (chelated OH), 1 645 (flavanone C=O), 1 580, 1 500 (aromatic C=C), 1 450, 1 410, 1 200, and 1 160 cm^{-1}; δ_H (60 MHz; $CDCl_3$) 1.43 (6 H, s, Me_2C), 2.80 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.20 (1 H, dd, *J* 17 and 4 Hz, 3-H), 3.74 (3 H, s, OMe), 3.82 (3 H, s, OMe), 5.25 (1 H, m, 2-H), 5.60 (1 H, d, *J* 10 Hz, chromene H), 5.98 (2 H, s, 6- and 8-H), 6.30 (1 H, d, *J* 10 Hz, chromene H), 6.60 (1 H, d, *J* Hz, 5′- or 7′-H), and 6.78 (1 H, d, *J* 2 Hz, 7′- or 5′-H), and 12.08 (1 H, br s, disappeared on deuteriation, 5-OH); 382 (M^+, 55%), 381 (14), 368 (52), 367 (100), 354 (8), 353 (30), 254 (20), 201 (35), and 69 (90).

Dehydrogenation of Sigmoidin B Dimethyldihydropyran Derivative (**9**).—A suspension of compound (**9**) (300 mg) in dry benzene (200 ml) was treated with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (600 mg) dissolved in benzene. The suspension was refluxed for 4 h, the solvent was evaporated off, and the residue was chromatographed on silica gel. Elution with hexane–ether (7:3) yielded sigmoidin C (**10**) (72%), indistinguishable from the natural sample (t.l.c., i.r., and ^1H n.m.r.).

Antibacterial Activity.—Minimal inhibitory concentrations (MIC) of the test compounds were determined* by standard tube-dilution techniques against representative Gram-positive and -negative organisms, and fungi. The following results were obtained.

	Test organism		
Substance	*Staphylococcus aureus*	*Trichophyton mentagrophytes*	*Bacillus subtilis*
Sigmoidin A	20 r		20 r
Sigmoidin B	20 r		
Amphotericin B		0.25 r	
Ampicillin	0.06 r		0.06 r

r = μg ml^{-1}

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