Metabolites of the higher fungi. Part 28.[1] Globoscinic acid and globoscin, a labile acid–lactone system from *Xylaria globosa* and *Xylaria obovata*

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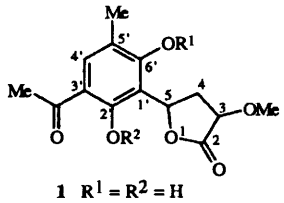
4-(3'-Acetyl-2',6'-dihydroxy-5'-methylphenyl)-4-hydroxy-2-methoxybutanoic acid (globoscinic acid) and 5-(3'-acetyl-2',6'-dihydroxy-5'-methylphenyl)-3-methoxy-2,3,4,5-tetrahydrofuran-2-one (globoscin) have been isolated as mixtures of optically active epimers from the culture media of the fungus *Xylaria globosa* and the fungus *Xylaria obovata*. Their structure determination by chemical and physical methods and their labile properties are described.

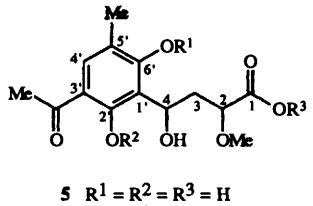
It is now believed that more than 500 individual xylaria species exist and that this is probably an underestimate of their true number. Combined with the other genera which constitute the family Xylariaceae the number of species is vast but our knowledge of their potential as a source of new metabolites and structures is confined to only a few species and most of these are confined to just two or three genera such as *Hypoxylon*, *Rosellinia* and *Xylaria*. We now report the isolation and characterisation of a labile acid–lactone system from the culture media of *Xylaria globosa* (Spring ex Fries) Mont. and *Xylaria obovata* (Berk.) Fr.

Various methods have been used by taxonomists to subclassify the members of the genus *Xylaria* and *X. globosa* has been assigned to the *X. polymorpha* group whose members exhibit considerable variation in their growth habit. *X. obovata* is considered closely related to *X. globosa*. *X. globosa* was collected from the Cameroon where it was found growing on a fallen log in a lowland evergreen forest, the two strains of *X. obovata* used originate from Malaya and Peru. Neither strain of the latter species produced 19,20-epoxycytochalasin Q_{hyp} or its deacetyl analogue, both of which are reported to be metabolites of a strain of *X. obovata* isolated from Ethiopia.[2] These two cytochalasins are metabolites produced by a number of different *Xylaria* species when cultured on artificial media.[3]

The fungi were surface cultured on 3% malt extract medium for 8 weeks. Both produced a radially growing mycelium which was at first colourless but later became black with a light brown slightly gelatinous underside. The mycelium supported copious (3 cm × 1 mm long) unbranched white tipped xylaria type stromata. Extraction of the culture medium with ethyl acetate gave a mixture of two compounds which we name globoscin **1** and globoscinic acid **5**; they were separated by chromatography. Both were detected on SiO_2 (TLC) by an orange colouration which developed with diazotised *p*-nitroaniline solution. The mycelium contained insignificant quantities of either metabolite.



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



5 $R^1 = R^2 = R^3 = H$
6 $R^1 = R^3 = Me$, $R^2 = H$
7 $R^1 = R^2 = H$, $R^3 = Et$

Table 1 ^1H NMR chemical shifts (relative to Me_4Si in $CDCl_3$) (multiplicity and *J* values) for the two diastereoisomers of globoscin

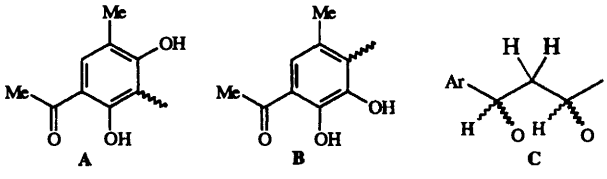
		(3*S*,5*S*) **15**	(3*S*,5*R*) **16**
2'	OH	13.14 (s)	13.10 (s)
3'	MeCO	2.56 (s)	2.55 (s)
4'	H	7.47 (s)	7.47 (s)
5'	Me	2.19 (d, 0.7)	2.20 (d, 0.7)
6'	OH	7.19 (s)	6.70 (s)
3	H	4.33 (dd, 8.25, 10.8)	4.21 (dd, 3.3, 7.3)
3	OMe	3.63 (s)	3.58 (s)
4	H_a	3.08 (ddd, 5.9, 8.25, 12.64)	2.70 (ddd, 3.3, 7.6, 13.9)
	H_b	2.40 (ddd, 10.6, 10.8, 12.64)	2.56 (ddd, 7.3, 7.4, 13.9)
5	H	5.95 (dd, 5.9, 10.6)	6.21 (dd, 7.6, 7.4)

Globoscin **1**, $C_{14}H_{16}O_6$, mp 160–165 °C, is optically active and can be recrystallised from ethyl acetate; it is insoluble in light petroleum and toluene, is sparingly soluble in chloroform and is soluble in ethanol with slow decomposition. The IR spectrum shows a chelated OH absorption at 3300–2800 cm^{-1} and carbonyl absorptions at 1774 and 1628 cm^{-1}. The former is indicative of the presence of either a phenolic ester or a γ-lactone and the latter of a strongly chelated carbonyl.

With diazomethane globoscin gives a monomethyl ether **2**, $C_{15}H_{18}O_6$, and acetylation with acetic anhydride and pyridine over 24 h produces a mixture of a monoacetate **3**, $C_{16}H_{18}O_7$ and diacetate **4**, $C_{16}H_{20}O_8$. In the methyl ether and monoacetate the positions of the hydroxy and carbonyl absorptions remain as in globoscin but in the diacetate the hydroxy absorption is missing and the carbonyl absorption at 1628 cm^{-1} now appears at 1680 cm^{-1}.

Globoscin as isolated occurs as a 1:1.4 mixture of two diastereoisomers. In the ^1H NMR spectrum ($CDCl_3$) the aromatic protons give a single resonance at δ 7.47 (Table 1), all the other protons show individual resonances, which are assignable to each stereoisomer by their unequal intensities. The differences in the resonance position of several of the structurally related protons in the two isomers is quite appreciable. This is clearly seen in the ^1H–^1H COSY spectrum, where two distinct spin systems can be elucidated.

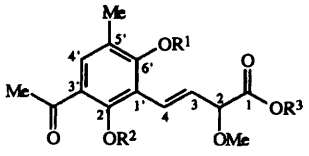
In the ^{13}C NMR spectra ($CDCl_3$) there are 26 resonances: these comprise isolated singlets at δ 202.9 and 26.28 each associated with two carbon atoms and twelve other pairs associated with six quaternary, three methine, one methylene, one methoxy and one methyl carbon atoms. The quaternary carbonyl pair near δ 174 identify the γ-lactone. The presence of only one aromatic proton in the ^1H NMR spectrum requires the presence of one methine and five quaternary aromatic carbons. These occur as two pairs of quaternary carbons attached to oxygen near δ 161 and 159, a methine pair near δ 132 and three quaternary pairs between δ 118–115. The remaining six carbons comprise two methines attached to oxygen between δ 77 and 72 (two pairs), a methoxy occurring as a well separated pair at δ 58.51 and 68.19, a methylene near δ 35 (one pair), a methyl attached to a carbonyl group at δ 26.28 (a single resonance pair) and the aromatic methyl near δ 15.4 (one pair). The ^1H NMR spectrum is dominated by two strongly hydrogen-bonded protons near δ 13.1, which together with the IR data confirms the presence of carbonyl group(s) hydrogen bonded to hydroxy groups. The aromatic protons at δ 7.47 are apparently uncoupled, however in the ^1H–^1H COSY spectrum these protons indicate coupling to the methyl pair near δ 2.2. The latter pair each show a 0.7 Hz coupling indicative of allylic coupling. This places the methyl group *ortho* to the aromatic proton. A ^1H–^1H NOESY spectrum revealed through space non-scalar coupling between the aromatic proton(s) at δ 7.47 and the methyl group(s) near δ 2.2 and the acetyl methyl group(s) at δ 2.5, which indicates the **A** or **B** substitution pattern



on the aromatic ring. The connectivities of the remaining groups which constitute the lactone ring were established from the ^1H–^1H COSY spectrum. The single proton resonance at δ 2.70 couples to a proton signal buried under the acetyl signal at δ 2.56. These two protons are non equivalent methylene protons because they both show a ^{13}C–^1H COSY correlation to the methylene carbon resonance at δ 35.35. Both protons couple to the *O*-methine proton at δ 4.21 (dd, *J* 7.3 and 3.3) and to the *O*-methine doublet of doublets (a pseudotriplet) at δ 6.21 (*J* 7.6 and 7.4) to give the spin system **C**. An analogous spin system can be traced for the other isomer, the proton resonating at δ 3.08 couples to that at δ 2.40 and both of these couple to the doublet of doublets signals at δ 4.33 and 5.95. The linkage of the aromatic ring *via* the *O*-methine carbon carrying the proton resonating at δ 6.21 (or 5.95) explains the lowfield positions of these methine protons.

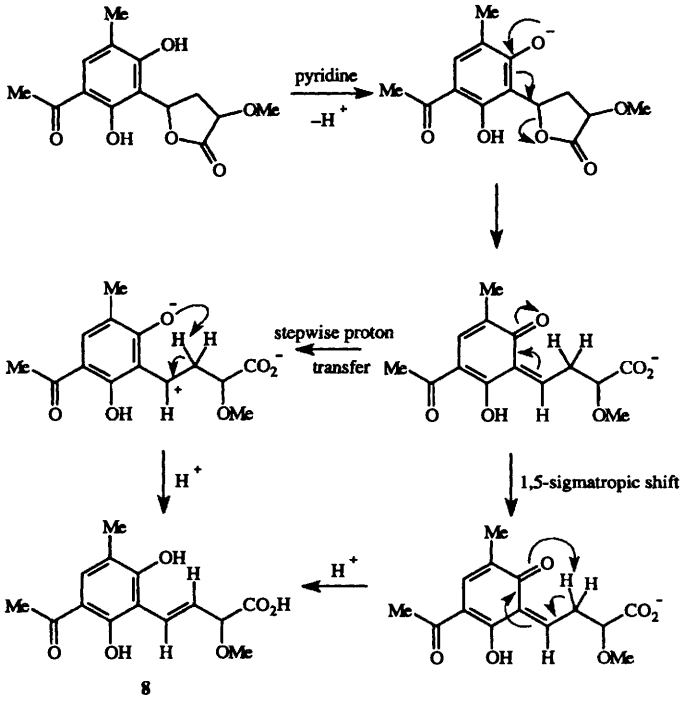
The monomethyl ether **2** prepared by methylating globoscin with diazomethane is a mixture of two separable gummy isomers which can be related to their parents by the similarities in their ^1H and ^{13}C NMR spectra. The mono-acetate **3** produced in admixture with the diacetate **4** by acetylating with acetic anhydride and pyridine is an optically inactive mixture of two epimers however, the diacetate is a single isomer and is optically active ($[\alpha]_D$ +9); all the ^1H and ^{13}C NMR signals are singlets and the *O*-acetyl groups resonate together. Acetylation of globoscin with acetic anhydride and sodium acetate produces a separable mixture of two diacetates. One of these is the same as the diacetate described above. The other was obtained as a gum with single signals which identify it as the diacetate of the other globoscin isomer.

Globoscin decomposes slowly in pyridine. After 12 h the ^{13}C NMR spectrum is complex with many additional signals but after 5 days the decomposition is complete; the double signals of the parent have disappeared and have been replaced by just 14 singlets. The aromatic methyl, acetyl and ring proton signals remain unchanged but two unsaturated methines now occur and the methylene and one of the methines attached to oxygen have disappeared. The new lowfield methines occur as *trans* coupled olefinic doublet of doublets at δ 7.84 (*J* 1.2 and 16.2) and 7.66 (*J* 6.4 and 16.2). The latter is adjacent to, and the former shows allylic coupling to an oxymethine doublet of doublets at δ 4.87 (*J* 1.2 and 6.4); this proton position is surprisingly similar to that of the oxymethine lactone proton in globoscin. Significantly, the IR lactone carbonyl absorption in globoscin at 1774 cm^1 has been replaced by an acid absorption at 1725 cm^{-1} which is shifted to 1745 cm^{-1} in **9** after methylation with diazomethane. These changes to yield the acid **8** can be



8 $R^1 = R^2 = R^3 = H$
9 $R^1 = R^3 = Me$, $R^2 = H$
10 $R^1 = R^2 = R^3 = Me$

explained by base-induced alkyl fission of the lactone followed by a sequential proton rearrangement. The process presumably involves the intermediate formation of a quinomethane (Scheme 1). Alternatively, the acid **8** could be formed *via* a 1,5



8

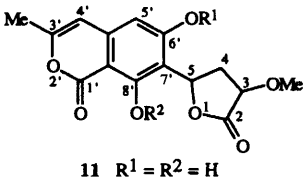
Scheme 1

sigmatropic rearrangement of the *o*-quinonoid intermediate. Significantly, monomethylation of globoscin at the 6′-hydroxy position prevents this isomerisation from taking place. In the FLOCK spectrum, which shows $^2J_{C-H}$ and $^3J_{C-H}$ correlations: the C-4 olefinic signal at δ 7.84 correlates to the quaternary phenolic 6′- and 2′-aromatic carbons at δ 162.46 and 162.86 and to the 2-*O*-methine carbon at δ 83.76 which, in turn, correlates to the OMe protons at δ 3.56. The correlations to the 6′- and 2′-

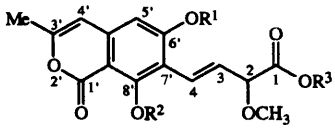
phenolic carbons establishes the attachment of the original ring between the two phenolic hydroxy groups as in **A**.

The more polar globoscinic acid **5**, $C_{14}H_{18}O_7$, M^+ 294, $\nu_{max}(CHCl_3)/cm^{-1}$ 3300–2500, 1719 and 1628; $[\alpha]_D^{23}$ −1.5 (*c* 1.0 in $CHCl_3$) was obtained as a gum comprising a mixture of two isomers; one was obtained pure but the second could not be completely separated from the first. Methylation with diazomethane resulted in partial lactonisation and formation of a mixture of the two isomeric globoscin monomethyl ethers and a mixture of isomeric ester ethers **6**, $C_{16}H_{22}O_7$, *m/z* 326, $\nu_{max}(CHCl_3)/cm^{-1}$ 1735 and 1628. Acetylation with acetic anhydride and pyridine causes spontaneous lactonisation and the formation of only the epimeric globoscin monoacetate mixture. The ^1H NMR spectrum of globosinic acid is similar to that of globoscin; the additional broad signals centred at δ 10.25 and 5.5 which disappear on addition of D_2O correspond to the acid and the C-4 secondary hydroxy group. The ^{13}C NMR spectrum of the diastereo mixture unlike that of globoscin, has only 16 resonances; only two of these occur as pairs near δ 79 and 69 and correspond to the asymmetric *O*-methine carbons found in globoscin. This hydroxy acid exists in equilibrium in aqueous solution with the lactone globoscin. After isolation and in the absence of water the acid undergoes a spontaneous slow lactonisation and after a few days the gummy isolate solidifies to yield globoscin. Crystallisation of globoscin from ethanol overnight results in partial formation of the hydroxy ester **7**.

To date the occurrence of only one other analogue of globoscin has been reported in the literature. Canescin **11** is



11 $R^1 = R^2 = H$
12 $R^1 = Me$, $R^2 = H$
13 $R^1 = R^2 = Ac$

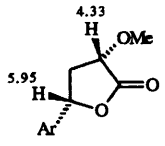


14 $R^1 = R^3 = Me$, $R^2 = H$

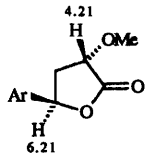
produced by *Aspergillus malignum* and *Penicillium canescens*[3] and its structure was determined by Birch *et al.*,[4,5] who subsequently carried out a biosynthetic study, which suggested a mixed origin for the isocoumarin and the lactone portions of the molecule.[6] The former was considered to originate from the acetate malonate pathway and the latter from succinate or fumarate; C-5 was apparently methionine derived and its exact synthetic role in the lactone biosynthetic pathway was unclear. Staunton *et al.*[7,8] re-examined the biosynthetic origin and fate of the C-5-methyl and using [$Me^{13}C^2H_3$]methionine, sodium [1,2-$^{13}C_2$]- and [1-^{13}C, 2-$^2H_3$]-acetate and sodium [2,3-$^{13}C_2$]- and [2-^{13}C, 2-$^2H_2$]-succinate were able to establish that the C-5 originates from methionine and is oxidised to formyl prior to succinate incorporation.

Globoscin differs from canescin in the aromatic part of the molecule. The chelated isocoumarin system of the latter is replaced by an aromatic ring bearing a chelated acetyl group, derived presumably from a tetraacetyl polyketide chain. In both compounds the attachment position of the furanone ring is flanked by two hydroxy groups. Like globoscin, canescin exists as a mixture of diastereoisomers however, in the ^1H NMR spectrum only the aliphatic methoxy signal occurs as a doublet in the parent, the monomethyl ether and the diacetate; Birch was only able to separate the diacetate into the individual isomers. Unlike globoscin, canescin does not appear to occur with the hydroxy acid in the culture medium. Although Birch and Staunton both used [$^2H_5$]pyridine as solvent in their ^1H and ^{13}C NMR studies on canescin they did not report the occurrence of any structural change in this solvent; presumably either none occurred or a change was given insufficient time to be noticeable. Canescin and globoscin both produce an unsaturated mono ester ether **14** and **6** on methylation with methyl iodide and potassium carbonate. The product from globoscin produced under identical conditions is the same as that formed by the action of diazomethane on the pyridine derived unsaturated acid. However, the unsaturated ester from canescin is reported to be the *cis*-isomer (*J* 6.0 Hz) with no interaction between the C-2 *O*-methine proton and the C-3 olefinic proton, whereas that from globoscin by both methods is *trans* (*J* 16 Hz) and the coupling to the C-2 *O*-methine proton is 6.4 Hz. Birch attributed the absence of coupling between the C-2 *O*-methine and the adjacent unsaturated proton to steric factors, but it is difficult to understand how such differences can arise from two so similar structures.

Stereochemical studies carried out by Birch *et al.*[2,3] established the configuration of the asymmetric carbon carrying the methoxy group in the γ-lactone ring as *S* in both of the canescin isomers. This was based on the exclusive formation of (*S*)-methoxyglutaric acid on ozonolysis and meant that the different stereoisomers must occur due to a difference at the C-5 lactone carbon. In the same way globoscin also exclusively yields (*S*)-methoxyglutaric acid on ozonolysis proving that the differences at C-5 give rise to the observed isomers. An ^1H–^1H NOESY spectrum of globoscin identifies the configuration of the C-5 lactone carbon in the two isomers relative to that at C-3.



15 *3S,5S*



16 *3S,5R*

In isomer **15** a through-space interaction occurs between the C-3 methine proton at δ 5.95 and the C-5 methine at δ 4.33; this establishes the 3*S*,5*S* configuration for this isomer. No such correlation occurs between the δ 4.22 and δ 6.21 protons in isomer **16** which is therefore established as 3*S*,5*R*. Although the biosynthetic pathway has not been determined it seems probable that globoscin and globoscinic acid join the very small number of metabolites where a methionine-derived methyl (C-5) is further alkylated. The occurrence of significant quantities of canescin in the fungus mycelium and the absence of the hydroxy acid in the medium is the reverse of the occurrence of globoscin and globoscinic acid and points to a possible difference in the final stages of formation of the lactone ring in the two compounds.

Experimental

Mps were determined on a Kofler hot-stage apparatus and are uncorrected, IR spectra on either a Perkin-Elmer 681 or a Nicolet 205 spectrophotometer, mass spectra (FAB) using 3-nitrobenzyl alcohol as matrix on an AEI MS 902 spectrometer and optical rotations on a Perkin-Elmer 141 polarimeter. $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}. Extracts were dried over Na_2SO_4 and the metabolites were detected on TLC with diazotised *p*-nitroaniline solution. Column chromatography was carried out using Merck Kieselgel GF_{254} or for flash chromatography, Fluka Kieselgel 60 (230–400) mesh. Ether refers to diethyl ether.

^1H and ^{13}C NMR spectra, using tetramethylsilane as internal standard, were determined at 270 or 67.8 MHz, respectively, with a JEOL GX 270 spectrometer fitted with a dual 5 mm C/H probe. ^1H NMR spectra (270 MHz) were acquired with 32K

data points over a spectrum width of 3001.2 Hz. Carbon atom types were established in the ^{13}C NMR spectrum by employing a combination of broad-band proton-decoupled and distortionless enhancement by polarisation transfer (DEPT) experiments with 32K data points over a spectrum width of 17 605.6 Hz. [$^2J_{C-H}$ and $^3J_{C-H}$] ^{13}C–^1H correlations were established by using the FLOCK pulse sequence of Reynolds *et al.*,[9] ^1H–^1H correlations were by double quantum filtered COSY. Other details are as published.[1]

Assignments were established by employing a combination of 1D and 2D NMR experiments. 2-Dimensional spectra were acquired and processed by standard JEOL software; ^1H–^1H correlations by double quantum-filtered COSY (VDQFN), resolution 2.93 Hz in the *f*1 and *f*2 domains, PW1 = PW2 = π/2; [$^1J_{C-H}$] ^{13}C–^1H correlations (VCHSHF), resolution *f*2 17.19 Hz and *f*1 5.9 Hz, pulse delay 1, 2 or 3 s, J_{C-H} 140 Hz; and [$^2J_{C-H}$ and $^3J_{C-H}$] ^{13}C–^1H correlations were established using the FLOCK pulse sequence of Reynolds *et al.*,[9] resolution *f*2 17.19 Hz and *f*1 5.9 Hz, pulse delay 1, 2 or 3 s, Δ^1 86.5 ms and Δ^2 46.5 ms, or Δ^1 44 ms and Δ^2 24 ms.

Globoscin 1 and globoscinic acid 5

The fungus *X. globosa* (KC 105) was surface cultured on 3% malt extract (20 dm^3) at 24 °C in Thompson bottles (2 dm^3) each containing 1 dm^3 of medium. The mycelium was removed by filtration through muslin. The brown culture filtrate was extracted three times with ethyl acetate and the extract was dried and evaporated to yield a brown gum (4 g); this was shown by TLC in the solvent system toluene–ethyl acetate–acetic acid (50:49:1) to consist of two main components. A solution of the gum (0.9 g) in the above solvent system (15 cm^3) was separated by flash chromatography on a column of SiO_2 (4 × 25 cm). Eluents were collected in 50 cm^3 fractions.

Tubes 1–5. Evaporation of the solvent gave *globoscin* **1** (71 mg) as colourless granules which crystallised from ethyl acetate as colourless needles consisting of a mixture of two diastereoisomers, mp 160–165 °C (Found: C, 60.0; H, 5.8. $C_{14}H_{16}O_6$ requires C, 60.0; H, 5.75%); $[\alpha]_D^{22}$ +23 (*c* 1.0 in acetone); *m/z* 280 (M^+), (FAB) $(M + Na)^+$ 303; ν_{max}(KBr)/cm^{-1} 3600–2500, 1774 and 1628; λ_{max}(dioxane)/nm 222, 233, 276 and 327 (ε/dm^3 mol^{-1} cm^{-1} 11 683, 8009, 8476 and 4441); (3*S*,5*R*)-*globoscin* **16**, δ_C($CDCl_3$) 202.91 (3′-*C*OMe), 174.15 (C-2), 160.85 (C-2′), 159.08 (C-6′), 132.63 (C-4′), 115.80 (C-5′), 113.27 (C-3′), 111.16 (C-1′), 76.17 (C-5), 74.12 (C-3), 58.17 (3-OMe), 35.38 (C-4), 26.28 (3′-CO*Me*) and 15.43 (5′-Me); (3*S*,5*S*)-*globoscin* **15**, δ_C($CDCl_3$) 202.92 (3′-*C*OMe), 173.65 (C-2), 160.47 (C-2′), 159.60 (C-6′), 132.55 (C-4′), 117.00 (C-5′), 112.94 (C-3′), 109.41 (C-1′), 75.67 (C-5), 72.87 (C-3), 58.51 (3-OMe), 34.86 (C-4), 26.29 (3′-CO*Me*) and 15.44 (5′-Me); see Table 1 for the δ_H data.

Tubes 9–12. Evaporation of the solvent afforded *globoscinic acid* **5**, $C_{14}H_{18}O_7$, as a gummy mixture of two isomers (2*S*,4*S* and 2*S*,4*R*) (148 mg), *m/z* 298 (M^+); $[\alpha]_D^{23}$ −1.5 (*c* 1.0 in $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3440, 3300–2500, 1719 and 1628; δ_H($CDCl_3$) 12.99 and 12.89 (2 H, s, 2′-OH), 10.25 (2 H, br s, CO_2H), 7.38 and 7.37 (2 H, s, 2 × 4′-H), 5.66–5.57 (2 H, m, 2 × 4-H), 5.50 (2 H, br s, 2 × 4-OH), 4.21–4.17 (2 H, m, 2 × 2-H), 3.55 and 3.52 (6 H, s, 2 × 2-OMe), 2.52 and 2.51 (6 H, s, 2 × 3′-*Me*CO), 2.37–2.25 (4 H, m, 2 × 3-H_2) and 2.13 and 2.15 (6 H, s, 2 × 5′-Me); δ_C($CDCl_3$) 202.77 (2 × 3′-*C*OMe), 175.75 and 175.70 (C-1), 162.28 and 162.24 (C-2′), 159.33 (2 × C-6′), 131.52 (2 × C-4′), 117.73 and 117.65 (C-5′), 112.17 (2 × C-3′), 112.01 and 111.94 (C-1′), 79.49 and 78.76 (C-2), 69.36 and 68.38 (C-4), 58.73 and 58.57 (2-OMe), 37.68 (2 × C-3), 26.12 (2 × 3′-*Me*CO) and 15.42 (2 × 5′-Me).

Tubes 25–32. Evaporation of the solvent afforded (2*S*,4*S*)-*globoscinic acid* **5** as a brown gum (62 mg), *m/z* 298 (M^+), $[\alpha]_D^{23}$ −92.5 (*c* 1.0 in $CHCl_3$); δ_H($CDCl_3$) 13.01 (1 H, s, 2′-OH), 10.25 (1 H, br s, CO_2H), 7.38 (1 H, d, *J* 0.7, 4′-H), 5.64 (1 H, dd, *J* 4.4 and 7.7, 4-H), 5.50 (1 H, br s, 4-OH), 4.17 (1 H, dd, *J* 7.5 and 7.0, 2-H), 3.54 (3 H, s, 2-OMe), 2.52 (3 H, s, 3′-*Me*CO), 2.28–2.36 (2 H, m, 3-H_2) and 2.13 (3 H, s, 5′-Me); δ_C($CDCl_3$) 202.75 (3′-*C*OMe), 175.91 (C-1), 162.32 (C-2′), 159.29 (C-6′), 131.51 (C-4′), 117.70 (C-5′), 112.18 (C-3′), 112.16 (C-1′), 79.09 (C-2), 68.79 (C-4), 58.44 (2-OMe), 37.49 (C-3), 26.13 (3′-CO*Me*) and 15.41 (5′-Me).

Acetylation of globoscin

***(a)* With pyridine and acetic anhydride.** A mixture of globoscin (50 mg), acetic anhydride (2 cm^3) and pyridine (1 drop) was set aside overnight and then was poured into cold water (15 cm^3) and set aside at 5 °C for 3 h. The resulting brown solution was extracted with ether (20 cm^3 × 3); evaporation of the washed and dried combined ether layers yielded a brown gum which was shown by TLC in the solvent system toluene–ethyl acetate–acetic acid (50:49:1) to be a mixture of two components identifiable as dark spots under UV light; these were separated by PLC. The more mobile component yielded a gum which gave needles from EtOH of *globoscin monoacetate* **3**, comprising a mixture of (3*S*,5*R*) and (3*S*,5*S*) isomers, mp 125–126 °C (Found: C, 59.7; H, 5.7. $C_{16}H_{18}O_7$ requires C, 59.6; H, 5.6%); *m/z* 322 (M^+); ν_{max}(KBr)/cm^{-1} 1778, 1638 and 1617; ν_{max}-($CHCl_3$)/cm^{-1}, 1780–1770 and 1645; δ_H($CDCl_3$) 12.88 and 12.83 (2 H, s, 2 × 2′-OH), 7.63 and 7.62 (2 H, s, 2 × 4′-H), 5.78–5.86 (2 H, m, 2 × 5-H), 4.23–4.33 (2 H, m, 2 × 3-H), 3.62 and 3.59 (6 H, s, 2 × 3-OMe), 2.87 (1 H, ddd, *J* 6.4, 8.8 and 12.8, 4-H_a), 2.70 (1 H, ddd, *J* 6.2, 8.1 and 13.9, 4-H_{aa}), 2.62 and 2.63 (6 H, s, 2 × 3′-*Me*CO), 2.43–2.54 (2 H, m, 4-H_b and 4-H_{bb}), 2.36 and 2.34 (6 H, s, 2 × 6′-OAc) and 2.13 (6 H, d, *J* 0.7, 2 × 5′-Me).

(3S,5R)-*Globoscin monoacetate* **3**, δ_C($CDCl_3$) 204.02 (3′-*C*OMe), 174.90 (C-2), 168.32 (6′-OAc), 159.95 (C-2′), 152.88 (C-6′), 132.71 (C-4′), 127.63 (C-5′), 121.72 (C-3′), 117.84 (C-1′), 76.26 (C-5), 72.41 (C-3), 58.24 (3-OMe), 34.52 (C-4), 26.81 (3′-CO*Me*), 20.50 (6′-OAc) and 16.02 (5′-Me); (3S,5S)-*globoscin monoacetate* **3**, δ_C($CDCl_3$) 203.94 (3′-*C*OMe), 174.58 (C-2), 168.32 (6′-OAc), 159.65 (C-2′), 153.81 (C-6′), 133.02 (C-4′), 127.63 (C-5′), 122.51 (C-3′), 117.62 (C-1′), 75.96 (C-5), 69.93 (C-3), 58.50 (3-OMe), 34.10 (C-4), 26.81 (3′-CO*Me*), 20.64 (6′-OAc) and 15.94 (5′-Me). Evaporation of the eluate containing the lower band gave needles from EtOH of (3S,5R)-*globoscin diacetate* **4**, mp 112–114 °C (Found: C, 59.4; H, 5.6. $C_{18}H_{20}O_8$ requires C, 59.3; H, 5.5%); $[\alpha]_D^{23}$ +9.20 (*c* 1.0 in $CHCl_3$); *m/z* 364 (M^+); ν_{max}($CHCl_3$)/cm^{-1} 1780–1770 and 1680; δ_H($CDCl_3$) 7.69 (1 H, s, 4′-H), 5.49 (1 H, dd, *J* 6.6 and 10.6, 5-H), 4.26 (1 H, dd, *J* 8.8 and 10.6, 3-H), 3.59 (3 H, s, 3-OMe), 2.81 (1 H, ddd, *J* 6.6, 8.8 and 12.8, 4-H_a), 2.53 (3 H, s, 3′-*Me*CO), 2.40 (1 H, ddd, *J* 10.6, 10.6 and 12.8, 4-H_b), 2.34 (3 H, s, 2′- or 6′-OAc), 2.33 (3 H, s, 2′- or 6′-OAc) and 2.19 (3 H, s, 5′-Me); δ_C($CDCl_3$) 196.73 (3′-*C*OMe), 174.33 (C-2), 169.55 (OAc), 168.09 (OAc), 150.95 (C-6′), 145.79 (C-2′), 132.85 (C-4′), 129.91 (C-5′), 128.85 (C-3′), 124.64 (C-1′), 75.73 (C-5), 70.35 (C-3), 58.66 (3-OMe), 34.87 (C-4), 29.02 (3′-COMe), 21.02 (OAc), 20.57 (OAc) and 16.35 (5′-Me).

***(b)* With acetic anhydride and sodium acetate.** A mixture of globoscin (200 mg), sodium acetate (400 mg) and acetic anhydride (2 cm^3) was refluxed for 45 min and then cooled, poured into water and set aside overnight at 5 °C. Extraction (× 3) with ether and evaporation of the ether yielded a pale brown gum which consisted of two components which were separated by PLC as before. The top band gave (3S,5S)-*globoscin diacetate* **4**, as a gum (71 mg), *m/z* 364 (M^+), $[\alpha]_D^{22}$ −1.0 (*c* 1.0 in $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 1780–1770 and 1680; δ_H($CDCl_3$) 7.69 (1 H, d, *J* 0.7, 4′-H), 5.78 (1 H, t, *J* 7.7, 5-H), 4.06 (1 H, dd, *J* 3.3 and 6.6, 3-H), 3.55 (3 H, s, 3-OMe), 2.52 (3 H, s, 3′-*Me*CO), 2.51–2.45 (1 H, m, 4-H_a), 2.47–2.34 (1 H, m, 4-

H_b), 2.33 (3 H, s, 2'- or 6'-OAc), 2.32 (3 H, s, 6'- or 2'-OAc) and 2.19 (3 H, s, 5'-Me); $\delta_C(CDCl_3)$ 196.85 (3'-*C*OMe), 173.82 (C-2), 169.35 (OAc), 168.02 (OAc), 150.43 (C-2'), 145.57 (C-6'), 132.46 (C-4'), 129.74 (C-5'), 129.06 (C-3'), 125.03 (C-1'), 75.93 (C-5), 73.44 (C-3), 58.21 (3-OMe), 36.46 (C-4), 29.04 (3'-CO*Me*), 20.98 (OAc), 20.48 (OAc) and 16.36 (5'-Me). The lower band gave a gum (107 mg) which gave needles from ethanol of (3S,5R)-*globoscin diacetate* **4**, mp 112–114 °C; identical with the product produced with acetic anhydride and pyridine.

Globoscin 6'-methyl ether 2

A suspension of globoscin in ether was treated with an ethereal solution of diazomethane for 4 h. The ether was evaporated to yield a pale brown gum which was shown by TLC in the solvent system toluene–ethyl acetate–acetic acid (50:49:1) to comprise a mixture of two isomers. Separation by PLC in the same solvent system gave firstly (3S,5R)-6'-*methoxyglobоscin* **2**, *m/z* 294 (M^+); $[\alpha]_D^{18}$ +3.3 (*c* 1.0 in $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3300–2500, 1774 and 1645; $\delta_H(CDCl_3)$ 12.88 (1 H, s, 2'-OH), 7.57 (1 H, d, *J* 0.7, 4'-H), 6.10 (1 H, dd, *J* 5.1 and 9.1, 5-H), 4.35 (1 H, dd, *J* 4.7 and 8.8, 3-H), 3.78 (3 H, s, 6'-OMe), 3.61 (3 H, s, 3-OMe), 2.74–2.81 (1 H, m, 4-H_a), 2.48–2.56 (1 H, m, 4-H_b), 2.60 (3 H, s, 3'-CO*Me*) and 2.26 (3 H, d, *J* 0.73, 5'-Me); $\delta_C(CDCl_3)$ 203.71 (3'-*C*OMe), 175.45 (C-2), 163.39 (C-2'), 160.61 (C-6'), 133.67 (C-4'), 127.63 (C-5'), 121.97 (C-3'), 116.43 (C-1'), 76.66 (C-5), 71.81 (C-3), 61.72 (6'-OMe), 58.25 (3-OMe), 34.42 (C-4), 26.66 (3'-CO*Me*) and 15.91 (5'-Me). The lower band yielded as a gum (3*S*,5*S*)-6'-methoxyglobоscin **2**, $\delta_H(CDCl_3)$ 12.84 (1 H, s, 2'-OH), 7.59 (1 H, d, *J* 0.7, 4'-H), 5.91 (1 H, dd, *J* 7.7 and 9.2, 5-H), 4.34 (1 H, dd, *J* 9.5 and 9.3, 3-H), 3.81 (3 H, s, 6'-OMe), 3.63 (3-OMe), 2.80–2.60 (2 H, m, 4-H_2), 2.59 (3 H, s, 3'-CO*Me*) and 2.27 (3 H, d, *J* 0.7, 5'-Me); $\delta_C(CDCl_3)$ 203.56 (3'-*C*OMe), 174.83 (C-2), 164.36 (C-2'), 160.75 (C-6'), 134.18 (C-4'), 122.08 (C-5'), 118.76 (C-3'), 116.31 (C-1'), 76.24 (C-5), 69.95 (C-3), 61.63 (6'-OMe), 58.15 (3-OMe), 33.14 (C-4), 26.67 (3'-CO*Me*) and 15.97 (5'-Me).

Acetylation of globoscinic acid

A mixture of the globoscinic acid **5** (50 mg), acetic anhydride (2 cm^3) and pyridine (1 drop) was set aside at room temperature overnight. The mixture was poured into water and set aside at 5 °C for 5 h. The resulting brown solution was extracted with ether, the extract dried and evaporated to yield a gum, which gave needles from EtOH of *globoscin monoacetate* **3**, mp 125–126 °C, identical with the product described above.

(2*S*,4*S*)-Globoscinic acid **5** (120 mg) was similarly acetylated to give a solid (78 mg) which recrystallised from alcohol as colourless needles of (3S,5S)-*globoscin monoacetate* **3** (56 mg), mp 125–126 °C; *m/z* 322 (M^+), $[\alpha]_D^{20}$ +30.4, $\delta_H(CDCl_3)$ 12.83 (1 H, s, 2'-OH), 7.63 (1 H, d, *J* 0.74, 4'-H), 5.83 (1 H, dd, *J* 6.6 and 10.6, 5-H), 4.29 (1 H, dd, *J* 8.8 and 10.6, 3-H), 3.62 (3 H, s, 3-OMe), 2.87 (1 H, ddd, *J* 6.6, 8.8 and 12.8, 4-H_a), 2.62 (3 H, s, 3'-CO*Me*), 2.39–2.51 (1 H, m, 4-H_b), 2.34 (3 H, s, 6'-OAc) and 2.12 (3 H, s, 5'-Me); $\delta_C(CDCl_3)$ 203.94 (3'-*C*OMe), 174.58 (C-2), 168.32 (6'-OAc), 159.65 (C-2'), 153.81 (C-6'), 133.02 (C-4'), 127.63 (C-5'), 122.51 (C-3'), 117.62 (C-1'), 75.96 (C-5), 69.93 (C-3), 58.50 (3-OMe), 34.10 (C-4), 26.81 (3'-CO*Me*), 20.64 (6'-OAc) and 15.94 (5'-Me).

Methylation of globoscinic acid 5

A solution of globoscinic acid in ether was treated with an ethereal solution of diazomethane. After removal of the ether the residual gum was separated into four fractions by PLC in the above solvent system. The two most mobile fractions constituted the two globoscin 6'-methyl ethers **2** described above. The two lower fractions yielded: *methyl* (2S,4R)-6'-*methoxyglobоscinate* **6**, as a gum, *m/z* 326 (M^+); $\nu_{max}(CHCl_3/cm^{-1})$ 3300–2500, 1735 and 1640; $\delta_H(CDCl_3)$ 12.99 (1 H, s, 2'-OH), 7.50 (1 H, d, *J* 0.7, 4'-H), 5.20–5.35 (1 H, br m, 4-H), 3.78–3.89 (1 H, m, 2-H), 3.81 (3 H, s, 6'-OMe), 3.77 (3 H, s, 2-OMe), 3.42 (3 H, s, CO_2Me), 2.53–2.59 (1 H, m, 3-H_a), 2.59 (3 H, s, 3'-COMe), 2.25 (3 H, s, 5'-Me) and 2.16–2.24 (1 H, m, 3-H_b); $\delta_C(CDCl_3)$ 204.02 (3'-*C*OMe), 173.15 (C-1), 162.34 (C-2'), 160.58 (C-6'), 132.21 (C-4'), 123.82 (C-5'), 122.33 (C-3'), 116.40 (C-1'), 78.28 (C-2), 75.96 (C-4), 69.93 (6'-OMe), 58.24 (2-OMe), 51.99 (CO_2Me), 39.71 (C-3), 26.70 (3'-CO*Me*) and 16.04 (5'-Me). The (2*S*,4*S*)-isomer of **6** was similarly obtained as a gum, *m/z* 326 (M^+); $\nu_{max}(CHCl_3)/cm^{-1}$ 3300–2500, 1735 and 1640; $\delta_H(CDCl_3)$ 13.02 (1 H, s, 2'-OH), 7.48 (1 H, s, 4'-H), 5.26 (1 H, dt, *J* 2.6 and 10.6, 4-H), 4.23 (1 H, dd, *J* 2.6 and 10.6, 2-H), 3.79 (3 H, s, 6'-OMe), 3.75 (3 H, s, 2-OMe), 3.48 (3 H, s, CO_2Me), 2.49–2.56 (1 H, ddd, *J* 2.6, 10.6, 14.3, 3-H_a), 2.59 (3 H, s, 3'-CO*Me*), 2.24 (3 H, s, 5'-Me) and 1.85–1.95 (1 H, ddd, *J* 2.6, 10.6 and 14.3, 3-H_b); $\delta_C(CDCl_3)$ 204.08 (3'-*C*OMe), 173.66 (C-1), 162.05 (C-2'), 160.57 (C-6'), 134.18 (C-4'), 124.31 (C-5'), 122.55 (C-3'), 116.29 (C-1'), 77.49 (C-2), 64.66 (C-4), 61.27 (6'-OMe), 58.60 (2-OMe), 51.95 (CO_2Me), 40.39 (C-3), 26.67 (3'-CO*Me*) and 15.99 (5'-Me).

Pyridine rearrangement product

A solution of globoscin (1 g) in pyridine (10 cm^3) was set aside for 5 days. The solution was evaporated to dryness to yield a brown gum which yielded plates of (3E)-4-(3'-*acetyl*-2',6'-*dihydroxy*-5'-*methylphenyl*)-2-*methoxybut*-3-*enoic acid* **8** (0.74 g), mp 165–166 °C from ethyl acetate (Found: C, 59.7; H, 5.8. $C_{14}H_{16}O_6$ requires C, 60.0; H, 5.75%); *m/z* 280 (M^+), FAB (M^+ + Na) 303; $\nu_{max}(CHCl_3)/cm^{-1}$ 3400–3000, 1725 and 1628; λ_{max}(EtOH)/nm 257, 295 and 336 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 25 500, 13 350 and 6160); $\delta_H(C_5D_5N)$ 14.14 (1 H, s, 2'-OH), 10.80 (1 H, br s, CO_2H), 7.84 (1 H, dd, *J* 1.2 and 16.2, 4-H), 7.66 (1 H, dd, *J* 6.4 and 16.2, 3-H), 7.56 (1 H, d, *J* 0.7, 4'-H), 4.86 (1 H, dd, *J* 1.2 and 6.4, 2-H), 3.56 (3 H, s, 2-OMe), 2.50 (3 H, s, 3'-CO*Me*) and 2.35 (d, *J* 0.7, 5'-Me); $\delta_C(C_5D_5N)$ 203.5 (3'-*C*OMe), 173.85 (C-1), 162.97 (C-2'), 162.46 (C-6'), 132.51 (C-4'), 130.05 (C-3), 124.15 (C-4), 116.92 (C-5'), 113.16 (C-3'), 112.48 (C-1'), 83.72 (C-2), 57.1 (2-OMe), 26.24 (3'-CO*Me*) and 17.44 (5'-Me).

Methyl (3*E*)-4-(3'-acetyl-2'-hydroxy-6'-methoxy-5'-methylphenyl)-2-methoxybut-3-enoate 9

The acid **8** (50 mg) was treated with an ethereal solution of diazomethane and the mixture set aside. After 2 h the ether was evaporated to yield a pale brown gum which yielded pale yellow silky needles of **9** (32 mg), mp 56 °C, from aqueous ethanol (× 3) (Found: C, 62.4; H, 6.6. $C_{16}H_{20}O_6$ requires C, 62.3; H, 6.5%); $[\alpha]_D^{23}$ −92 (*c* 1.1 in $CHCl_3$); $\delta_H(CDCl_3)$, 13.11 (1 H, s, 2'-OH), 7.49 (1 H, d, *J* 0.7, 4'-H), 6.95 (1 H, dd, *J* 16.0 and 0.7, 4-H), 6.78 (1 H, dd, *J* 16.0 and 7.0, 3-H), 4.44 (1 H, dd, *J* 7.0 and 0.7, 2-H), 3.78 (3 H, s, 6'-OMe), 3.73 (3 H, s, 2-OMe), 3.47 (3 H, s, CO_2Me), 2.59 (3 H, s, 3'-CO*Me*) and 2.24 (3 H, s, 5'-Me); $\delta_C(C_5D_5N)$ 204.85 (3'-*C*OMe), 171.48 (C-1), 163.62 (C-2'), 161.64 (C-6'), 133.0 (C-4'), 130.1 (C-3), 124.22 (C-4), 122.26 (C-5'), 118.13 (C-3'), 116.61 (C-1'), 82.62 (C-2), 60.22 (6'-OMe), 57.21 (2-OMe), 51.98 (CO_2Me), 26.70 (3'-CO*Me*) and 15.63 (5'-Me).

The same compound was prepared by refluxing a mixture of globoscin (100 mg), dry anhydrous potassium carbonate (1.0 g) and methyl iodide (10.0 g) in acetone (30 cm^3) for 1 h. The mixture was filtered, evaporated and the residue recrystallised from aqueous ethanol (× 3) to yield the above ether (42 mg).

Methyl (3*E*)-4-(3'-acetyl-2',6'-dimethoxy-5'-methylphenyl)-2-methoxybut-3-enoate 10

A mixture of globoscin (1.0 g), potassium carbonate (1.0 g) and methyl iodide (6 cm^3) in acetone (30 cm^3) was refluxed for 10 h. The mixture was filtered and the filtrate evaporated. The

residual gum (0.89 g) was dissolved in ether and the solution washed with water, dried and evaporated to yield an orange oil which was separated by PLC (20 × 20 cm) × 5 in the solvent system toluene–ethyl acetate–acetic acid (75:25:1) into two components which were detected as purple bands under UV light. The upper more mobile component crystallised from aqueous alcohol to yield the ether **9** (32 mg) described above. The less mobile component **10** was obtained as a pale yellow oil (530 mg), $\nu_{max}(CHCl_3)/cm^{-1}$ 1748 and 1677; *m/z* 308 (M^+, 2%), 276 (8), 249 (16) and 217 (100); $\delta_H(CDCl_3)$, 7.43 (1 H, s, 4′-H), 6.89 (1 H, dd, *J* 16.1 and 1.1, 4-H), 6.65 (1 H, dd, *J* 16.1 and 7.0, 3-H), 4.48 (1 H, dd, *J* 7.0 and 1.1, 2-H), 3.80 (3 H, s, 6′-OMe), 3.69 (3 H, s, 2-OMe), 3.49 (6 H, s, 2′-OMe and CO_2Me), 2.62 (3 H, s, 3′-CO*Me*) and 2.26 (3 H, s, 5′-Me).

Ozonolysis of globoscin

Globoscin (0.71 g) was methylated for 11 h as described above and the oily product, without purification, was dissolved in alcohol (25 cm^3) and hydrogenated at room temperature and atmospheric pressure in the presence of palladium–charcoal (50 mg, 5%) until hydrogen was no longer absorbed (2 h). The solution was filtered and evaporated to yield an almost colourless oil, which was dissolved in acetic acid (20 cm^3) and then ozone was gently bubbled through the solution for 10 h. Hydrogen peroxide (10 cm^3, 30%) was added, the solution set aside overnight and then evaporated. The colourless oil was dissolved in ether (10 cm^3) and washed with water (×3) to remove water soluble acids. An ethereal solution of diazomethane was added to the dried ether layer and after 30 min the ether layer was evaporated and the oil chromatographed by PLC in the solvent system hexane–ethyl acetate (85:15) to yield methyl (+)-(*S*)-α-methoxyglutarate, $[\alpha]_D^{23}$ +34 (*c* 1.0 in $CHCl_3$) (lit.,[4] +36.3); *m/z* 131 (M^+ − 59, 41%), 115 (18), 99 (27), 75 (7), 71 (100), 55 (23) and 44 (40).

Acknowledgements

We thank the SERC and the Royal Botanic Gardens, Kew for providing the Case studentship to M. A., and Zeneca plc for gifts of solvent and for microanalyses. A voucher specimen and culture of *X. globosa* (KC 105) is retained at Kew.

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Paper 5/02383A
Received 13*th April* 1995
Accepted 11*th May* 1995