Biosynthesis of Mangostin. Part 1: The Origin of The Xanthone Skeleton[1]

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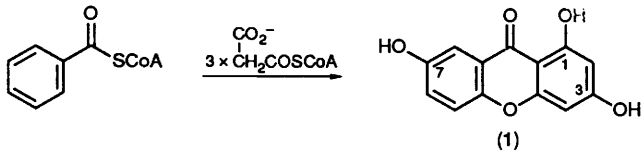
The biosynthesis of mangostin (**3**) has been studied by wick-feeding of ^{14}C- and ^{13}C-labelled precursors to young *Garcinia mangostana* plants. Radioactive mangostin was isolated as 3,6-di-*O*-methylmangostin (**4**), which was subsequently degraded to phloroglucinol and isovaleric acid to aid location of the label. Although results from feeding of ^{14}C-labelled precursors suggested two alternative malonate–shikimate routes to compound (**3**), experiments with ^{13}C-labelled compounds clearly demonstrated that mangostin (**3**) originates from a C_6C_1 unit (benzoate) and three C_2 units (malonates). [1,2,3-$^{13}C_3$]malonic acid was incorporated solely into ring A of compound (**4**) and two different arrangements of C_2 units in compounds (**17a** and **b**) were evident, indicating that mangostin (**3**) derives *via* ring closure of a symmetrical intermediate (**18**).

Unlike xanthones from fungi, which have been shown to be wholly acetate-derived,[2] higher plant xanthones[3] exhibit oxygenation patterns which indicate that they originate by a combination of the acetate (ring A) and shikimate (ring B) pathways. Birch and Donovan[4] noted two acetate–shikimate combinations by which the $C_6C_1C_6$ xanthone skeleton could be formed, *viz.* either C_6C_1 + 3 C_2 or C_6C_3 + 2 C_2. Indeed, both combinations have been identified in three studies on the biogenesis of higher plant xanthones.[5–7] There remains, however, much speculation regarding the nature of the shikimate moiety as well as the mode of xanthone formation.[8]

Two investigations have been carried out on the biosynthesis of the 1,3,7-trioxygenated xanthones of *Gentiana lutea* L. (Gentianaceae). Floss and Rettig found that 98% of the label from [1-^{14}C]acetate was located in the 1,3-dioxygenated ring of gentisin (**1**).[5] Gupta and Lewis obtained a similar result on feeding of [2-^{14}C]acetate.[6] [U-^{14}C]Phenylalanine was also well incorporated, and the absence of activity in the phloroglucinol ring of gentisin (**1**) led the authors to suggest the involvement of a phenylalanine-derived C_6C_1 unit (Scheme 1).



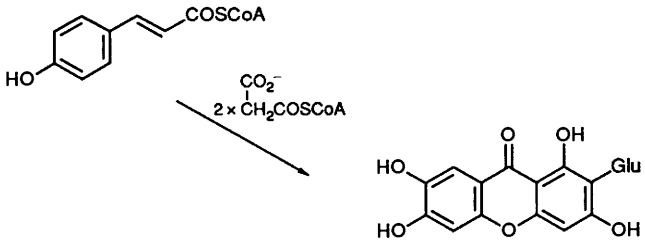
(1)

Scheme 1.

The biosynthesis of the *C*-glucosylxanthone mangiferin (**2**) in *Anemarrhena asphodeloides* (Lilaceae) has been thoroughly studied by Fujita and Inoue.[7] They found that mangiferin was formed from *p*-hydroxycinnamate and two malonate units (Scheme 2). The involvement of a C_6C_3 unit confirmed earlier proposals[8a,9] on the close biogenetic relationship between mangiferin (**2**) and flavonoids.

Mangostin (**3**) is the major xanthone constituent of the mangosteen tree, *Garcinia mangostana* L. (Guttiferae).[10] Although Guttiferae xanthones differ from those found in the Gentianaceae in that they are often *C*-prenylated,[11] xanthones from the two families exhibit many of the same oxygenation patterns and may well have a common origin. The ready availability of *G. mangostana* seedlings in Singapore prompted us to investigate the biosynthesis of mangostin (**3**).

Studies with ^{14}C-Labelled Precursors.—^{14}C-Labelled precursors were wick-fed to the upper aerial parts of the seedlings for periods of 7 days. After being dried, the plants were extracted with benzene and the crude extract was methylated to give 3,6-di-*O*-methylmangostin (**4**). Methylation not only gave an easily purified product, but also augmented the yield, since two minor xanthones, β- and γ-mangostin (**5**)[12] and (**6**),[13] were also converted into the triether (**4**). To optimize the yield of compound (**4**), the methylation was monitored by TLC until the disappearance of 3,6-di-*O*-methyl-γ-mangostin (**7**), which was found to be slow to methylate due to the hindered 7-hydroxy group. In addition to compound (**4**), four other methylated xanthones, (**8**), (**9**), (**12**), and (**13**), were isolated. These are the methyl ethers of the natural products (**10**),[14] (**11**),[14] (**14**),[15] and (**15**),[16] respectively.



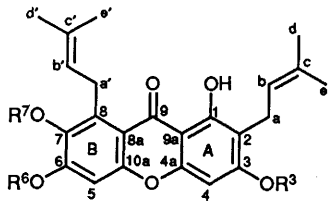
(2)

Scheme 2.

Initial attempts to degrade 3,6-di-*O*-methylmangostin (**4**) using hot conc. aq. potassium hydroxide failed to rupture the xanthone nucleus. However, in addition to recovered starting material and β-mangostin (**5**), a xanthone isomeric with compound (**4**) was isolated. ^1H and ^{13}C NMR spectra indicated structure (**16**) for this xanthone, with the C-8 side-chain double bond conjugated with ring B.

Degradation of compound (**4**) in molten alkali was more successful. Phloroglucinol and isovaleric acid were isolated, the latter as its *S*-benzylisothiouronium salt, and their specific activities were determined. The specific activity of the salt led to only an approximation of the total side-chain activity as the two 3-methylbut-2-enyl substituents of compund (**4**) may have different activities and one may be preferentially cleaved. Attempts to isolate a fragment from ring B in sufficient yield for radioactive counting were unsuccessful.

The results from the feeding of various ^{14}C-labelled precursors are shown in Table 1. [2-^{14}C]Malonic acid was incorporated to the extent of 1% and as expected the label



(3) $R^3 = R^6 = H$, $R^7 = Me$
(4) $R^3 = R^6 = R^7 = Me$
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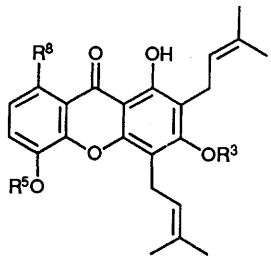
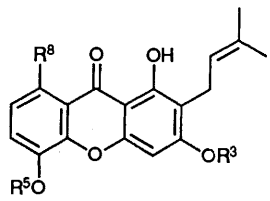
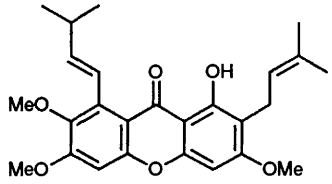


(8) $R^3 = R^5 = Me$, $R^8 = H$
(9) $R^3 = R^5 = Me$, $R^8 = OH$
(10) $R^3 = R^5 = R^8 = H$
(11) $R^3 = R^5 = H$, $R^8 = OH$



(12) $R^3 = R^5 = Me$, $R^8 = H$
(13) $R^3 = R^5 = Me$, $R^8 = OH$
(14) $R^3 = Me$, $R^5 = R^8 = H$
(15) $R^3 = Me$, $R^5 = H$, $R^8 = OH$



(16)

Table 1. Experiments with ^{14}C-labelled precursors.

Precursor (μCi)	Sp. Act. (dpm mmol^{-1})	Yield (4) (mg)	Sp. Act. (4) (dpm mmol^{-1})	% Incorporation	% Activity in Ring A	% Activity in Side-chains
[2-^{14}C]Malonic Acid (50)	1.0×10^{11}	256	2.09×10^{6}	1.10	71	14
L-[1-^{14}C]Phenylalanine (50)	1.2×10^{11}	178	3.98×10^{4}	0.15	85	5
L-[U-^{14}C]Phenylalanine (50)	1.1×10^{12}	327	9.95×10^{5}	0.67	12	3.5
[3-^{14}C]Cinnamic acid (50)	1.2×10^{11}	270	2.39×10^{6}	1.32	1.4	<1
[3-^{14}C]Cinnamic acid (15)	4.5×10^{8}	256	7.06×10^{5}	1.24	1.6	
[2-^{14}C]Cinnamic acid (15)	4.5×10^{8}	232	5.13×10^{5}	0.82	93	
p-Hydroxy [2-^{14}C]-cinnamic-acid (15)	4.5×10^{8}	315	2.6×10^{4}	0.06		

primarily located in ring A of mangostin (**3**). Both [1-^{14}C]- and [U-^{14}C]-phenylalanine were less well utilized by the plant, and in both cases a proportion of the label was also detected in the phloroglucinol ring. [3-^{14}C]Cinnamic acid was a good precursor to mangostin (**3**) and as ring A was devoid of activity, the label was assumed to be present in the xanthone carbonyl carbon. [2-^{14}C]Cinnamic acid was similarly well incorporated and the label was almost totally present in the phloroglucinol ring. This result, together with those obtained from feeding of [^{14}C]phenylalanines, implied the incorporation of an intact C_6C_3 unit, pointing to a route similar to that found for mangiferin (**2**). However, in contrast to the biosynthesis of mangiferin (**2**), *p*-hydroxy[2-^{14}C]cinnamic acid was poorly incorporated, while [*carboxy*-^{14}C]benzoic acid was a very efficient precursor. This last result clearly pointed to the involvement of a C_6C_1 unit, as proposed for the biosynthesis of gentisin (**1**).

Studies with ^{13}C-Labelled Precursors.—In an attempt to differentiate between the C_6C_1 and C_6C_3 routes, three studies were carried out using ^{13}C-labelled precursors. To begin with, the ^{13}C NMR spectrum of compound (**4**) was fully assigned (Table 2). In particular, the two resonances at δ 155, ascribed to C-4a and C-10a, were ascertained by low-power irradiation of 4-H and 5-H.

Results from the incorporation of [^{14}C]cinnamic acid (Table 1) were used to estimate the mass of [$^{13}C_2$]cinnamic acid required to be fed in order to produce detectable ^{13}C–^{13}C coupling[17] in the ^{13}C NMR spectrum of compound (**4**), and this was confirmed by a trial experiment in which cinnamic acid (30 mg) spiked with [^{14}C]cinnamic acid gave a specific incorporation of 0.25% (Table 3, Expt. 1). Accordingly, [2,3-$^{13}C_2$]cinnamic acid was prepared from [*carbonyl*-^{13}C]benzaldehyde and [2-^{13}C]malonate (both 99% enriched) and wick-fed to a plant together with [3-^{14}C]cinnamic acid to monitor the incorporation (Table 3, Expt. 2). The ^{13}C NMR spectrum of the isolated triether (**4**) did not show coupling between C-9 and C-9a even though the specific incorporation as calculated from

Table 2. ^{13}C NMR data for *Garcinia mangostana* xanthones and derivatives.

Carbon	(4)	(16)	(5)	(7)	(12)	(8)	(10)	(9)	(13)
1	159.6	159.6	159.8	159.8	159.4	159.9	157.7	158.4	158.9
2	111.3	111.3	111.5	111.3	111.9	113.5	106.7	113.9	112.5
3	163.2	163.2	163.5	163.3	164.3	163.8	160.8	164.6	165.1
4	88.5	88.6	88.8	88.5	90.0	117.1	110.5	117.7	90.4
5	98.1	98.3	101.5	96.9	148.1[d]	148.7[e]	146.4[f]	140.3	139.9
6	157.9	158.4	154.4[b]	152.7[c]	115.1	115.7	114.5	121.2	119.7
7	143.9	143.7	142.6	140.3	123.2	123.2	123.6	108.8	108.9
8	136.9	133.4	137.1	132.3	116.6	116.5	120.2	154.2[g]	154.0[h]
9	181.8	181.8	181.9	182.3	180.5	181.8	180.7	185.6	184.4
4a	154.9	155.0[a]	155.2[b]	155.2[c]	155.9	152.8	152.2	152.9[g]	156.1[h]
8a	111.8	111.9	112.4	112.0	121.4	121.1	120.7	108.0	108.1
9a	103.7	103.9	103.9	104.0	103.7	106.0	102.3	105.0	102.7
10a	155.2	154.7[a]	155.7[b]	154.7[c]	146.0[d]	146.6[e]	145.1[f]	146.1	145.4
a/a′	21.4, 26.2	21.3, 143.0	21.4, 26.5	21.4, 26.0	21.3,	22.6, 22.8	21.4, 21.6	22.6, 22.7	21.3
b/b′	122.5, 123.4	122.5, 120.9	122.3, 123.2	122.4, 122.6	122.1,	122.7, 122.8	122.5,	122.4, 122.5	121.8
c/c′	131.5	131.4 32.1	131.6 132.1	131.6	131.8	131.7, 131.8	130.9, 130.8	132.0	132.1
d/d′	25.9, 25.8	25.8, 22.8	25.8	25.8, 25.6	25.7	25.7	25.5, 25.6	25.7	25.8
e/e′	17.8, 18.2	17.8, 22.8	17.8, 18.3	17.8, 18.0	17.8,	17.8, 17.9	17.8	17.8, 17.9	17.8
OMe	55.7, 55.9, 60.8	55.7, 56.0, 59.8	55.8, 62.0	56.3, 57.8	56.0, 56.3	56.2, 61.8		57.6, 61.9	56.1, 57.2

[a–h] Assignments marked with same letter may be interchanged within a column.

Table 3. Experiments with ^{13}C-labelled cinnamic and benzoic acids.

	Precursor		Dimethylmangostin		
Precursor	Activity (μCi)	Weight (mg)	Yield (mg)	Sp. Act. (dpm mmol^{-1})	Dilution/ % Sp. Incorpn.
1 [3-^{14}C]cinnamate	13	30	215	3.66×10^5	400/0.25
2 [2,3-$^{13}C_2$]cinnamate	7	22	191	3.07×10^5	340/0.29
3 [*carboxy*-^{14}C]benzoate	17	25	181	4.24×10^6	45/2.2
4 [*carboxy*-^{13}C]benzoate	17	25	236	3.66×10^6	52/1.9, 2.5[a]

[a] Determined from ^{13}C NMR spectrum.[2b]

Table 4. Incorporation of [1,2,3-$^{13}C_3$]malonic acid.

Carbon	C-1	C-2	C-3	C-4	C-4a	C-9a
Chemical shift	159.6	111.3	163.2	88.5	154.9	103.7
Coupling constants (Hz)	73.9, 63.5	73.9, 70.8	70.2	69.6, 73.9	64.7, 74.5	64.1
%Enrichment[17]	0.30	0.37	0.33	0.38	0.34	0.41

^{14}C was 0.29%, sufficient for such coupling to be clearly visible had the precursor been incorporated intact.

A likely explanation for the observed incorporation of [1-^{14}C]phenylalanine and [2-^{14}C]cinnamic acid is that β-cleavage[18] of these compounds had occurred and that the two-carbon fragments lost were incorporated as [1-^{14}C]- and [2-^{14}C]-acetate, respectively, into the phloroglucinol ring of mangostin (**3**).

The high incorporation of [^{14}C]benzoic acid (Table 1) suggested it may be possible to detect the incorporation of a singly ^{13}C-labelled benzoate by ^{13}C NMR spectroscopy. A preliminary study with [^{14}C]benzoic acid (25 mg) gave a dilution of 45 (Table 3, Expt 3), low enough to produce measurable peak enhancement. [*carboxy*-^{13}C]Benzoic acid (99% enriched) was fed and the ^{13}C NMR spectrum of the resultant triether (**4**) displayed a carbonyl peak more than three times natural-abundance intensity, clearly establishing the origin of the xanthone carbonyl as well as ring B from benzoate. The specific incorporation calculated from the normalized peak intensities [2b] was 2.5%, while the result from ^{14}C measurements was 1.9% (Table 3, Expt. 4).

In a third ^{13}C study [1,2,3-$^{13}C_3$]malonic acid was fed to a plant to confirm that ring A was wholly derived from malonate. While the ^{13}C NMR spectrum of xanthone (**4**) isolated from the leaves of the plant was identical with a natural-abundance spectrum, the spectrum of compound (**4**) obtained from the plant stem displayed six resonances with satellite peaks (Figure 1, Table 4). These corresponded to the six ring A carbons. Four of the carbons showed two sets of satellites of equal intensity, indicating that the $^{13}C_2$ units were arranged in two different ways [structures (**17a** and **b**), Scheme 3] and that mangostin (**3**), like the fungal xanthone ravenelin,[2a] derives from a symmetrical intermediate, *e.g.* the benzophenone (**18**). The appearance of only one set of satellites about C-3 and C-9a is due to the similarity of their coupling constants with adjacent carbons.

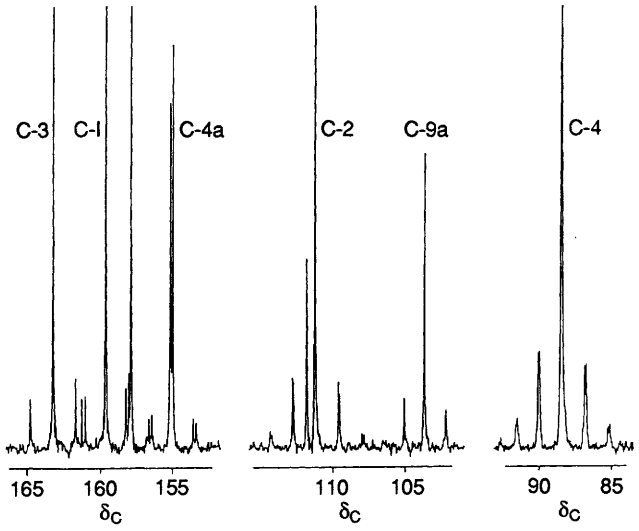
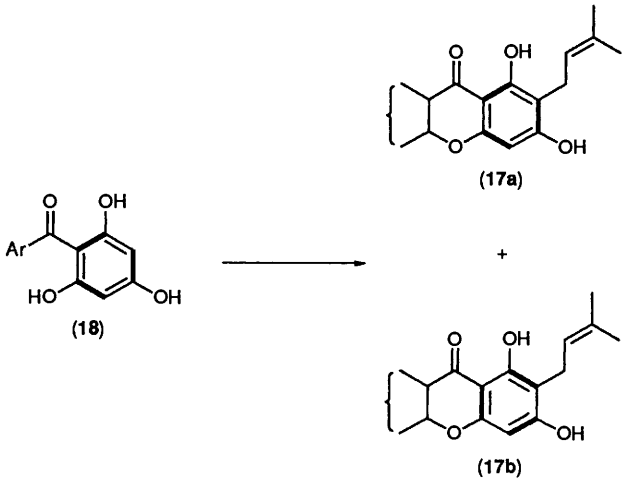


Figure 1. Proton-noise-decoupled ^{13}C NMR spectrum of dimethylmangostin (**4**) labelled by [1,2,3-$^{13}C_3$]malonic acid.



Scheme 3.

Lower intensity, more distant satellites are visible about C-2 and C-4. These are due to splitting of the main satellites due to the presence of molecules containing two adjacent $^{13}C_2$ units.[19] This was not unexpected as the plant was saturated with a highly enriched precursor. The percentage enrichment for each carbon was calculated from the ratio of the sum of the integrals of the main satellites and the integral of the central peak (Table 4).[17] There was no observable incorporation of $^{13}C_2$ units into the 3-methylbut-2-enyl side-chains of mangostin.

Experimental

General.—^1H and ^{13}C NMR (Table 2) spectra were recorded in $CDCl_3$ on a JEOL FX90Q instrument. UV (EtOH) and IR ($CHCl_3$) spectra were obtained using a Shimadzu UV-240 and a Perkin-Elmer 1301 spectrophotometer, respectively. Mass spectra were recorded on a VG Micromass 7035 instrument. Column chromatography was carried out using Merck Kieselgel 60 (63–200 μm), and flash chromatography according to the method of Still.[20] Preparative TLC (PLC) was performed using precoated Merck Kieselgel 60 plates of 0.5 or 2.0 mm layer thickness. Elemental analyses were carried out by the Microanalytical Laboratory, National University of Singapore.

Radioactivity measurements were made on a Beckmann LS 3801 counter using Omnifluor (New England Nuclear) (6 g) dissolved in toluene (1 l) and Triton X-100 (0.5 l) as the scintillant. ^{14}C-Labelled compounds were obtained from the following sources. Amersham: L-[1-^{14}C]phenylalanine; C.E.R.N. (France): L-[U-^{14}C]phenylalanine, [3-^{14}C]cinnamic acid; New England Nuclear: [2-^{14}C]malonic acid, [*carboxy*-^{14}C]benzoic acid; Sigma: [2-^{14}C]malonic acid. ^{13}C-labelled compounds were obtained as follows: Sigma: [*carboxy*-^{13}C]benzoic acid, [2-^{13}C]malonic acid; ICN Biomedicals: [*carbonyl*-^{13}C]benzaldehyde; and Isotech Inc. (Ohio): [1,2,3-$^{13}C_3$]malonic acid. *Garcinia mangostana* seedlings (1–2 years old) (30–50 cm tall) were obtained as required from the Primary Production Department, Ministry of National Development, Singapore. No special incubation procedures were used during feeding as the climate here is fairly constant throughout the year.

Synthesis of Labelled Compounds.—[2-^{14}C]Cinnamic acid was prepared from [2-^{14}C]malonic acid (110 μCi), malonic acid (52 mg, 0.5 mmol), and benzaldehyde (80 mg, 0.75 mmol) according to published procedure.[21] The product was recrystallized from aq. methanol (51 mg, 71%), m.p. 127–129 °C; specific activity 4.88×10^8 dpm mmol^{-1}.

p-Hydroxy[2-^{14}C]cinnamic acid was prepared from *p*-hydroxybenzaldehyde (75 mg, 0.61 mmol), [2-^{14}C]malonic acid (100 μCi), and malonic acid (55 mg, 0.53 mmol), according to the method of Adams and Bockstahler.[22] The product (61 mg, 70%) was recrystallized from methanol, m.p. 213–214 °C; specific activity 4.52×10^8 dpm mmol^{-1}. Synthetic [2-^{14}C]-cinnamic acid and commercial [3-^{14}C]cinnamic acid (15 μCi each) were diluted with inactive material to a specific activity of 4.5×10^8 dpm mmol^{-1} before feeding.

[2,3-$^{13}C_2$]Cinnamic acid was prepared in essentially the same way as [2-^{14}C]cinnamic acid, from 99.6% [2-^{13}C]malonic acid (100 mg, 0.95 mmol) and 99% [*carbonyl*-^{13}C]benzaldehyde (85 mg, 0.79 mmol); yield 75 mg (64%), m.p. 131 °C. The proton-noise-decoupled ^{13}C NMR spectrum of the product exhibited two doublets centred at δ 146.99 and 117.23. The intensities of the central uncoupled peaks were *ca.* 1.2% of the combined satellite intensities. Before feeding, this product was recrystallized from methanol containing [3-^{14}C]cinnamic acid (20 μCi).

Administration of Precursors.—Feeding experiments were conducted throughout the year with two or more plants being fed at the same time for comparison purposes.

(i) ^{14}C-Labelled precursors were wick-fed to intact plants at two points 5 cm apart halfway up the stem. A needle was used to bore the two wick-holes horizontally through the centre of the stem. Two moistened, non-mercerized cotton threads were passed through each hole and the ends of the threads were immersed in two vials (0.25 ml) attached on opposite sides of the stem just below each hole. A precursor dissolved in a suitable solvent (see below) was distributed among the four vials. As the vials emptied (1–3 days), water was added to wash in any remaining precursor, and washing was continued until a total of 7 days had elapsed. Where possible, precursors were dissolved in water. Benzoic and cinnamic acids were dissolved in various volumes and concentrations of aq. sodium hydroxide: [*carboxy*-^{14}C]benzoic acid and [3-^{14}C]cinnamic acid (50 μCi) (1.0 ml; 0.01M); other [^{14}C]cinnamic acids (15 μCi) (1.5 ml; 0.05M).

(ii) ^{13}C-Labelled precursors were similarly wick-fed but, owing to the larger solvent volumes, two additional feeding points were used near the top of the stem. All ^{13}C-labelled precursors were dissolved in aq. sodium hydroxide: [*carboxy*-^{13}C]benzoic acid (25 mg) (3.0 ml; 0.06M); [2,3-$^{13}C_2$]cinnamic acid (22 mg) (3.0 ml; 0.03M); [1,2,3-$^{13}C_3$]malonic acid (50 mg) (4.8 ml; 0.1M).

Isolation of Dimethylmangostin (**4**).—After the seven-day feeding period the plant stem was excised 2 cm below the lower

wick and the cutting was freeze-dried. The dry material (20–25 g) was cut into small pieces, homogenized in benzene, and then extracted with benzene in a Soxhlet apparatus for 48 h. The residue obtained after removal of solvent was methylated with dimethyl sulphate (0.4 ml, 4.2 mmol) in the presence of anhydrous potassium carbonate (0.8 g, 6.0 mmol) in refluxing acetone (100 ml) for *ca.* 5 h, until TLC (hexane–dichloromethane, 1:1) indicated the absence of 3,6-di-*O*-methyl-γ-mangostin (**7**) (R_F 0.32). After the usual work-up, the dark green residue was chromatographed on a column of silica gel (150 × 30 mm) and eluted initially with hexane–dichloromethane (2:1) to remove the orange carotenoid band. Further elution with hexane–dichloromethane (1:2) gave a yellow xanthone fraction. PLC of this fraction [four 2 mm plates; hexane–ethyl acetate (5:1), 2 developments] produced three bands. The middle, major band constituted compound (**4**) (150–350 mg), which was crystallized from methanol as pale yellow, woolly needles, m.p. 119–120 °C (lit.,[10] 121.5–122.5 °C); λ_{max}(log ε) 243 (4.45), 262 (4.53), 312 (4.37), and 348 nm (3.81); δ 1.69 (6 H, s), 1.81 (3 H, s), and 1.87 (3 H, s), (2 × =CMe_2), 3.35 (2 H, br d, a-H_2), 3.80, 3.89 and 3.95 (each 3 H, s, 3 × OMe), 4.13 (2 H, br d, a′-H_2), 5.1–5.4 (2 H, m, 2 × =CH), 6.29 (1 H, s, 4-H), 6.70 (1 H, s, 5-H), and 13.43 (1 H, s, 1-OH).

The less polar band was found to be a mixture of two xanthones. These were separated by flash chromatography [hexane–dichloromethane (4:1)] to give (i) 3,5-di-*O*-dimethyl-8-deoxygartanin (**8**) (40–90 mg), yellow microscopic needles, m.p. 117–118 °C (from MeOH) (lit., [23] 106–108 °C); λ_{max} 242 (4.48), 258 (4.50), 311 (4.01), and 375 nm (3.63); δ 1.69 (6 H, s), 1.81 (3 H, s), and 1.86 (3 H, s) (2 × =CMe_2), 3.40 and 3.51 (each 2 H, each d, 2 × CH_2), 3.83 and 3.91 (each 3 H, each s, 2 × OMe), 5.2–5.4 (2 H, m, 2 × =CH), 7.0–7.2 (2 H, m, 6- and 7-H), 7.68 (1 H, dd, 8-H), and 12.93 (1 H, s, 1-OH). Demethylation of compound (**8**) (400 mg) with hot morpholine–water (4:1; 15 ml)[13] and separation of the products by PLC [hexane–ethyl acetate (4:1)] gave 8-deoxygartanin (**10**) (64%), m.p. 164–165 °C (lit.,[14] 165.5 °C) with spectroscopic data comparable to those reported;[14] and (ii) 3,5-*di*-O-*methylgartanin* (**9**) (20–60 mg), bright yellow, matted needles, m.p. 166–167 °C (from MeOH) (Found: C, 70.6; H, 6.9%; M^+, 424.1891. $C_{25}H_{28}O_6$ requires C, 70.7; H, 6.65%; *M*, 424.1886); λ_{max} 239 (4.30), 262 (4.45), 278 (4.38), 350 (4.03), and 400 nm (3.53); δ 1.69 (6 H, s), 1.79 (3 H, s), and 1.86 (3 H, s) (2 × =CMe_2), 3.39 and 3.54 (each 2 H, each br d, 2 × CH_2), 3.81 and 3.90 (each 3 H, each s, 2 × OMe), 5.1–5.4 (2 H, m, 2 × CH), 6.66 and 7.22 (each 1 H, each d, *J* 8.9 Hz, 7- and 6-H), and 11.39 and 12.10 (each 1 H, each s, 8- and 1-OH); *m*/*z* (%) 424 (100), 409 (18), 381 (74), 370 (13), 369 (59), 353 (24), and 299 (15); di-*O*-methyl derivative, m.p. 82–83 °C (lit.,[14] 85 °C).

The most polar band was also a mixture. PLC [hexane–dichloromethane (2:1)] produced partial resolution, enabling isolation of a pure sample of the following two xanthones: 1,8-*dihydroxy*-3,5-*dimethoxy*-2-(3-*methylbut*-2-*enyl*)*xanthone* (**13**), which was crystallized from methanol as yellow needles, m.p. 178–181 °C (Found: C, 67.25; H, 5.65%; M^+, 356.1244. $C_{20}H_{20}O_6$ requires C, 67.4; H, 5.7%; *M*, 356.1260); λ_{max} 244 (4.41), 254 (4.41), 281 (4.42), 316 (4.10), 335 (4.07), and 390 nm (3.64); δ 1.65 and 1.75 (each 3 H, each s, =CMe_2), 3.26 (2 H, br d, CH_2), 3.89 and 3.95 (each 3 H, each s, 2 × OMe), 5.10 (1 H, br t, =CH), 6.44 (1 H, s, 4-H), 6.57 and 7.10 (1 H each, d, *J* 8.8 Hz, 7- and 6-H), and 11.23 and 11.98 (1 H, s, 8- and 1-OH); *m*/*z* (%) 356 (79), 341 (49), 314 (15), 313 (77), 302 (19), 301 (00), and 285 (28); and 1-hydroxy-3,5-dimethoxy-2-(3-methylbut-2-enyl)-xanthone (**12**) as small yellow needles from methanol, m.p. 166–167 °C (lit.,[15] 168–169 °C); δ 1.69 and 1.80 (each 3 H, each s, =CMe_2), 3.37 (2 H, br d, CH_2), 3.79 and 3.86 (each 3 H, each s, 2 × OMe), 5.24 (1 H, br t, =CH), 6.58 (1 H, s, 4-H), 7.1–7.3 (2 H, m, 6- and 7-H), 7.82 (1 H, dd, 8-H), and 12.89 (1 H, s, 1-OH).

3,6-*Di*-O-*methyl*-γ-*mangostin* (**7**).—This xanthone appeared on the PLC plates as an additional band, more polar than that of compounds (**12**) and (**13**), if methylation was incomplete. It crystallized as small, pale yellow *needles*, m.p. 192–194 °C (from MeOH) (Found: C, 70.7; H, 6.7%; M^+, 424.1891. $C_{25}H_{28}O_6$ requires C, 70.7; H, 6.65%; *M*, 424.1866); λ_{max} 243 (4.37), 262 (4.50), 313 (4.30), and 364 nm (3.75); δ 1.68 (6 H, s), 1.80 (3 H, s), and 1.85 (3 H, s) (2 × =CMe_2), 3.35 (2 H, br d, a-H_2), 3.89 and 3.98 (each 3 H, each s, 2 × OMe), 4.14 (2 H, br d, a′-H_2), 5.1–5.3 (2 H, m, 2 × =CH), 5.62 (1 H, s, D_2O exchangeable, 7-OH), 6.28 (1 H, s, 4-H), 6.68 (1 H, s, 5-H), and 13.52 (1 H, s, 1-OH); *m*/*z* (%) 424 (100), 382 (15), 381 (660), 369 (86), 368 (57), 367 (22), 354 (16), 353 (67), and 325 (47). Methylation with dimethyl sulphate–potassium carbonate gave compound (**4**).

Degradation of Dimethylmangostin (**4**).—In preliminary studies, following the method of Yamashiro,[24] using conc. aq. potassium hydroxide (25 g in 4 ml) at 220 °C, dimethylmangostin (**4**) (2.5 g) failed to dissolve, and a yellow solid was recovered by filtration. Besides unchanged starting material, two minor products were noted by TLC. These were separated by flash chromatography [hexane–dichloromethane (3:2)] and were identified as β-mangostin (**5**), m.p. 172–174 °C (lit.,[12] 175–176 °C), and 1-*hydroxy*-3,6,7-*trimethoxy*-8-[(E)3-*methylbut*-1-*enyl*]-2-(3-*methylbut*-2-*enyl*)*xanthone* (**16**), lemon yellow needles, m.p. 124–125 °C (from MeOH) (Found: C, 71.2; H, 6.9%; M^+, 438.2045. $C_{26}H_{30}O_6$ requires C, 71.2; H, 6.9%; *M*, 438.2042); λ_{max} 247 (4.59), 258sh (4.47), 314 (4.36), and 355sh nm (3.82); δ 1.12 and 1.21 (each 3 H, each s, d′- and e′-H_3), 1.68 and 1.79 (each 3 H, each s, d- and e-H_3), 2.53 (1 H, br m, c′-H), 3.33 (2 H, br d, a-H_2), 3.68, 3.89, and 3.97 (each 3 H, each s, 3 × OMe), 5.23 (1 H, t, b-H), 6.15 (1 H, dd, *J* 6.7 and 16.0 Hz, b′-H), 6.25 (1 H, s, 4-H), 6.68 (1 H, s, 5-H), 7.10 (1 H, dd, *J* 1.3 and 16.0 Hz, a′-H), and 13.48 (1 H, s, 1-OH); *m*/*z* (%) 438 (24), 396 (29), 395 (100), and 339 (18).

(ii) Radioactive dimethylmangostin (**4**) (81 mg) was diluted to 400 mg with inactive (**4**) and added in portions during 5 min to a nickel crucible containing a molten mixture of sodium and potassium hydroxides (1:1; 6 g) under nitrogen at 260 °C. The temperature was raised to 290–300 °C during 20 min. After a further 30–40 min, fairly vigorous effervescence set in and heating was stopped. The mixture was cooled under nitrogen, dissolved in cold water (50 ml), and carefully acidified to Congo Red with ice-cold, dil. sulphuric acid. The volatile components were removed by steam distillation, and the residual liquid was filtered and then subjected to continuous extraction with diethyl ether (150 ml) for 72 h. The organic layer was shaken briefly with water (15 ml), then dried (Na_2SO_4), and the solvent was removed under reduced pressure. PLC [hexane–ethyl acetate (1:1)] of the pale brown residue gave phloroglucinol, which was purified by sublimation (bath temp. 130–140 °C; 0.05 mmHg) to give crystals (15 mg), m.p. 211–213 °C (lit.,[25] 219 °C). The specific activity was determined and then the product was resublimed until a constant specific activity was attained.

Crude isovaleric acid was obtained by extraction (dichloromethane) of the steam distillate, and was purified as its *S*-benzylisothiouronium salt (25 mg), m.p. 153–155 °C (lit.,[25] 159 °C).

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