The Biosynthesis of Cucurbitacin B

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Summary Evidence is presented that is consistent with cucurbitacin B being biosynthesized *via* cycloartenol or parkeol but not through the intermediacy of lanosterol.

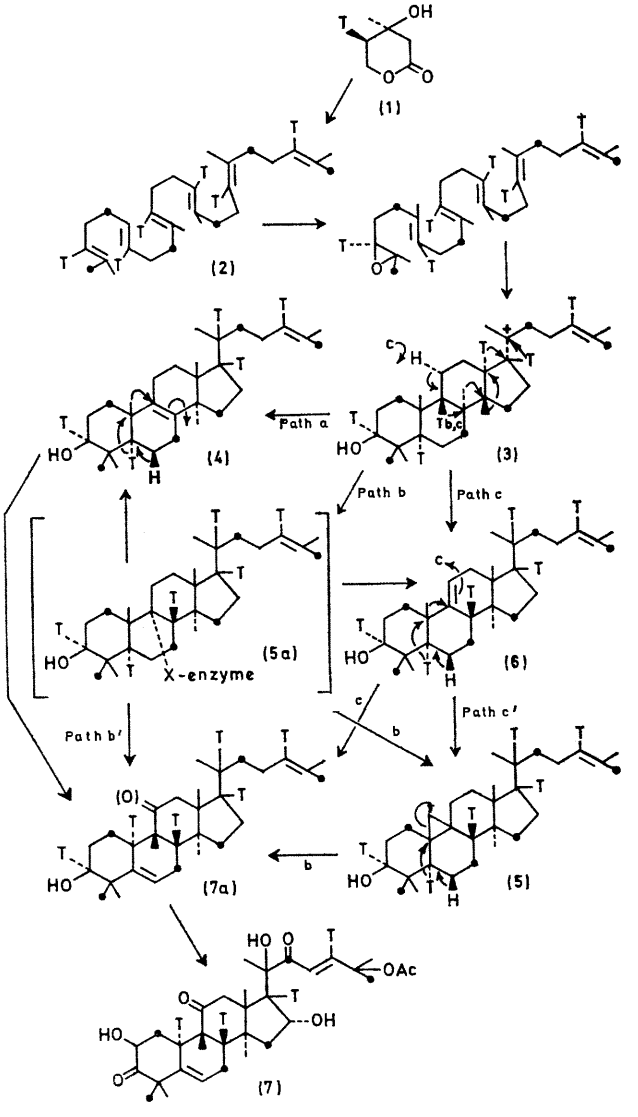
THE chemistry and properties of the cucurbitacins have been well documented;[1] in contrast, the mechanisms by which these novel compounds are biosynthesized remain unknown. Of particular interest is the unusual methyl substitution pattern of ring B, and we have therefore investigated the mechanism of elaboration of this ring by means of labelled precursors.

The biosynthesis of the cucurbitacins from [(4*R*)-4-$^3H_1$,2-^{14}C]mevalonic acid (MVA) **1**) can be expected to involve the initial formation of the cation (**3**); from this cation several routes are then possible (Scheme). Loss of the C-9β hydrogen, accompanied by the multiple migrations indicated will lead (path a) to lanosterol (**4**), which may then rearrange to give the cucurbitacin skeleton (**7a**). Such a sequence would entail the loss of tritium from C-9 with the result that, together with the loss of the two tritium atoms from C-3 and C-20, cucurbitacin B (**7**) would have a $^3H/^{14}C$ ratio of 3/6 relative to that of the original MVA or squalene (**2**). The intermediacy of lanosterol in mammalian biosynthesis is well precedented.[2]

An alternative route (path c) involves the loss of the C-11 proton from (**3**) to give parkeol (**6**), with retention of the C-9 trition through migration to C-8. The migration of the 10-methyl (C-19) may then be induced by the creation of an electron deficiency at C-9—such a deficiency could arise either by protonation or by formation and opening of a 9α, 11α-epoxide, for which there is chemical precedent.[3] This latter possibility is attractive as it would account for the C-11 oxygen function which is common to all known cucurbitacins. The cucurbitacin B resulting from such a scheme should then have a $^3H/^{14}C$ ratio of 4/6 that of the parent squalene.

The third major possibility (path b) invokes the intermediacy of cycloartenol (**5**), a substance of widespread occurence in the plant world, whose ubiquity[4] and demonstrated intermediacy[5] in phytosterol biosynthesis has led to suggestions[6] that it replaces lanosterol as a precursor of plant sterols. In this case the $^3H/^{14}C$ ratio in the derived cucurbitacin B would again be 4/6. However, the mode of formation of cycloartenol itself is relevant here: objections have been raised[7] to its formation directly from cation (**3**), and it has been postulated[7] that (**5a**) serves as an intermediate.[8] Such an intermediate, by elimination of the appropriate protons at C-6β, C-11β, or C-19, in concert with the appropriate migrations, could lead to any or all of (**4**), (**5**), (**6**), or (**7a**). For (**5**), (**6**), and (**7a**) the trition at C-8 would again be retained. Alternatively, cycloartenol may be formed *via* parkeol (path c').

We have been able to illuminate some of these pathways by means of incorporation of [(4*R*)-4-^3H,2-^{14}C]MVA into cucurbitacin B in squash. Thus the radicles of two-day-old Hubbard squash seedlings were soaked in a solution of sodium DL-[(4*R*)-4-$^3H_1$,2-^{14}C]mevalonate (1·7 mg, 23 μCi of ^{14}C, $^3H/^{14}C$ = 10·8) and the seedlings then incubated for



SCHEME

45 h in dilute Webb's medium[9] at 80° under strong light. Extraction of the seedlings for cucurbitacin B[10] then gave a chloroform extract which was chromatographed on silica gel ($CHCl_3$–EtOH, 97 : 3). Rechromatography of the cucurbitacin B band, followed by co-crystallization to constant specific activity with authentic material, give pure

cucurbitacin B, $^3H/^{14}C$ = 7·55, containing 1·73 × 10^5 d.p.m. of ^{14}C (0·76% incorporation of the active isomer of MVA).

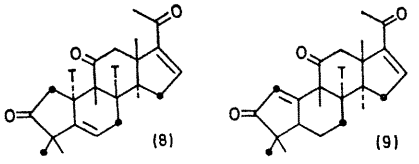
ν_{max} ($CHCl_3$) 1740, 1692, 1667, and 1592 cm^{-1}. The mass spectrum showed prominent peaks at *m/e* 354, 339, and 219,

TABLE

Compound	[4*R*-$^3H_1$,2-^{14}C]MVA experiment $^3H/^{14}C$ Ratio d.p.m.	atomic Found	atomic Theory	[2-$^3H_2$,2-^{14}C]MVA experiment $^3H/^{14}C$ Ratio d.p.m.	atomic Found	atomic Theory
Squalene (**2**)	11·00		6/6	7·25		12/6
Cycloartenol (**5**)	10·91	5·95/6	6/6			
Cucurbitacin B (**7**) ..	7·55	4·12/6	4/6 or 3/6	6·55	10·78/6	10/6
(**8**)	5·91	2·16/4	2/4 or 1/4			
(**9**)	3·77	1·37/4	1/4 or 0/4			

Saponification of the insoluble plant debris from the initial extraction procedure afforded in the usual way[10] a non-saponifiable extract which was acetylated and chromatographed on an alumina column (Woelm, grade III). Elution with petroleum gave squalene (**2**), purified twice by t.l.c.[11] and then passage through the thiourea clathrate complex,[12] having a $^3H/^{14}C$ ratio of 11·00 and a total activity of 2 × 10^4 d.p.m. of ^{14}C (0·06% from MVA). Subsequent elution yielded, after t.l.c. on $AgNO_3$-impregnated silica gel and co-crystallization to constant specific activity, cycloartenyl acetate (6 × 10^3 d.p.m. of ^{14}C; $^3H/^{14}C$ = 10·95).

In a duplicate experiment using sodium DL-[2-$^3H_2$,2-^{14}C]-mevalonate (1·4 mg, 28 μCi of ^{14}C, $^3H/^{14}C$ = 8·5) with slightly younger seedlings and an incubation time of 51 h, an incorporation of MVA into cucurbitacin B of 1·2% (based on the active isomer) was achieved. The yield of squalene ($^3H/^{14}C$ = 7·25 [13]) was again 0·06%.



The cucurbitacin B (**7**) derived from the [(4*R*)-4-$^3H_1$,2-^{14}C]MVA was then degraded in order to establish the location of the tritions and to check that the nuclear ratios had not arisen fortuitously due to randomization. Treatment with periodic acid,[14] followed by hot Na_2CO_3 gave, after purification by t.l.c. (silica gel, EtOAc : benzene 1 : 3) and recrystallization from ether, compound (**8**), $^3H/^{14}C$ = 5·91, m.p. 187—190° λ_{max} (EtOH) 239 nm (ε 8670), the last fragment being interpreted as arising from McLafferty-type transfer of the C-11 proton to the C-11 oxygen followed by retro-Diels–Alder fission of ring B.[15] Acid isomerization[14] of (**8**) gave (**9**), purified thrice by t.l.c. (silica gel, ethyl acetate : benzene 1 : 3), $^3H/^{14}C$ = 3·77, ν_{max} ($CHCl_3$) 1725, 1700, 1662, and 1595 cm^{-1}, λ_{max} (EtOH) 229 nm, *m/e* 354. This substance had lost 70% of the expected tritium activity. Insufficient material was available for crystallization, which may account for the high ratio.

From these results (see Table), the following conclusions may be drawn: (i) Sodium mevalonate is incorporated efficiently and in a non-random fashion into cucurbitacin B. (ii) Functionalization at C-2, C-6, C-16, and C-25 does not involve equilibration at the adjacent C-1, C-7, C-15, and C-26 positions. (iii) The protons at the C-3, C-20, and C-22 are lost in the oxidation at these centres, and do not migrate to any significant extent. (iv) Double-label ratios demonstrate that the protons at C-5 and C-9 of (**3**) migrate to C-10 and C-8, respectively, of cucurbitacin B. This result is inconsistent with the intermediacy of lanosterol (path a) or of a $\Delta^{5(10)}$ compound in cucurbitacin biosynthesis and is consistent with the other routes which may or may not involve parkeol or cycloartenol. (v) The mechanism of cycloartenol biosynthesis operating in squash is presumably analogous to that in potato,[7] since the pattern of mevalonate incorporation is the same.

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