Biosynthesis of Cantharidin: Evidence for the Specific Incorporation of C-4 and C-11′ of Farnesol

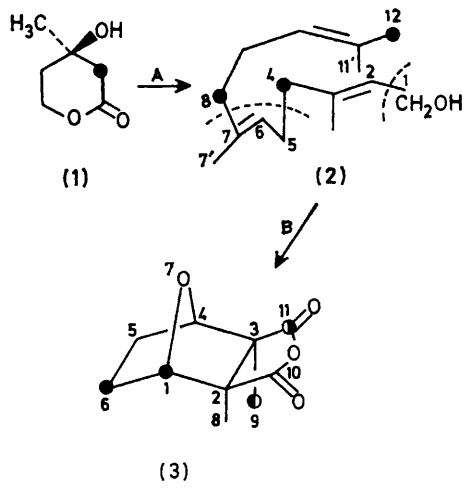
Wolf-Dietrich Woggon,* Suzanne A. Hauffe, and (the late) Hans Schmid

Organisch-chemisches Institut der Universität Zürich-Irchel, Winterthurerstrasse 190, 8057 Zürich, Switzerland

(*E,E*)-[11′,12-$^{3}H_{2}$,2,4-^{14}C]farnesol is specifically incorporated into cantharidin as shown by use of a new degradation involving a Diels–Alder/retro-Diels–Alder sequence; the incorporation of (*E,E*)-[11′-^{3}H,^{14}C,2-^{14}C]-farnesol (**14**) into cantharidin proceeds with 47% ^{3}H-retention whereby the 11′-^{14}C label is randomised at C-9 and C-11 in cantharidin and the ratio [2-^{14}C]/[11′-^{14}C] of (**14**) is retained indicating an intramolecular conversion of farnesol into cantharidin.

Various labelling studies have shown that mevalonate (**1**)[1] and farnesol (**2**)[2] are specific biogenetic precursors for cantharidin (**3**), the toxic defensive secretion of blister beetles (*Lytta vesicatoria*, *Meloidae*, *Coleoptera*), Scheme 1.

In order to add substantial evidence for bondbreaking between C-4 and C-5 of farnesol[3] in its conversion into cantharidin we report the specific incorporation of C-4 of farnesol. A straightforward synthesis of (*E,E*)-[4-^{14}C]farnesol



Scheme 1

Table 1. Degradation of [^{3}H,^{14}C]cantharidin (**8**), isolated from male *Lytta vesicatoria* after injection of (**7**).[a]

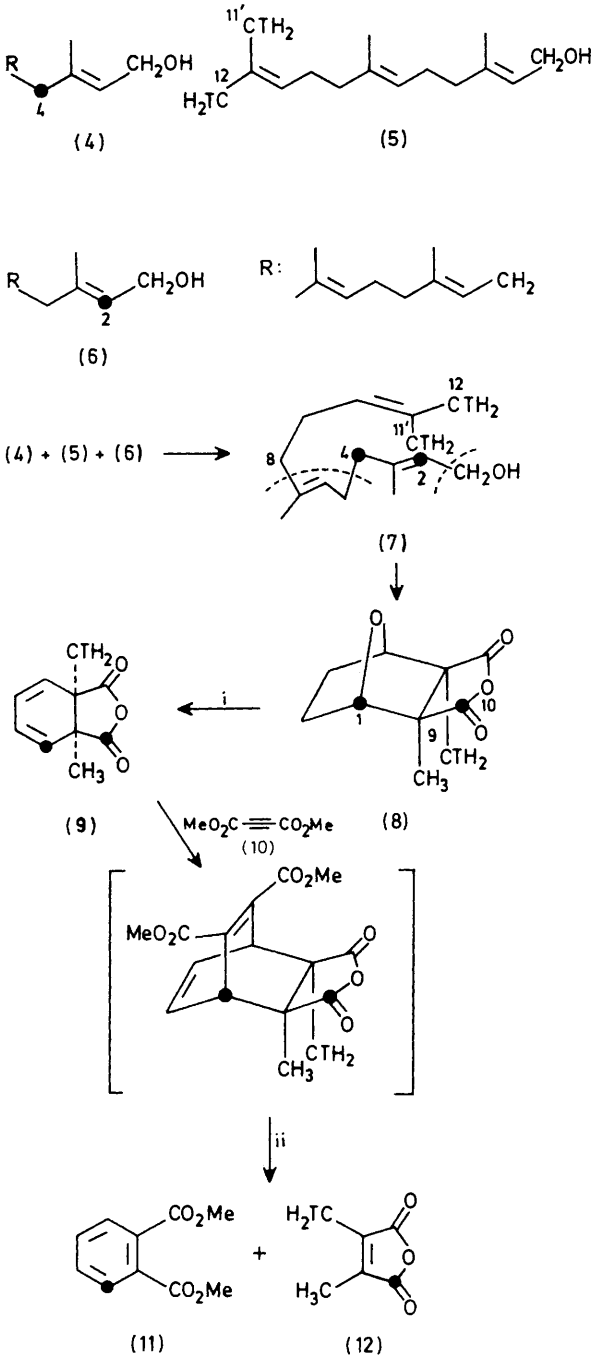
Compound[b]	^{3}H/^{14}C	^{3}H rel.%	^{14}C rel.%
(**7**)	3.13 : 1	50 at C-11′ 50 at C-12	32.4 at C-4 67.6 at C-2
(**8**)	1.60 : 1	100	100
CTH_2CO_2H[c]	∞	90.6	0
(**11**)	0	0	29.0
(**12**)	2.40 : 1	99.5	66.5

[a] 0.8 mmol of (**8**) were used for the degradation, displaying specific activities for ^{3}H of 7.96 × 10^5 d.p.m./mmol and for ^{14}C of 4.96 × 10^5 d.p.m./mmol. [b] Compounds (**7**) and (**11**) were counted in the form of their purified 3,5-dinitrobenzoate and *p*-tolylimide, respectively, CTH_2CO_2H as the *p*-bromophenacyl ester. [c] Sum of C-2, C-3, C-8, and C-9.

(**4**) was developed† giving (**4**) in 14% yield from diethyl [2-^{14}C]-malonate (NEN, BRD; sp. act. 3.6 mCi/mmol; purity 97%), exhibiting a specific activity of 1.92 mCi/mmol. For the preparation of the multiply labelled farnesol (**7**), (**4**) was mixed with (*E*,*E*)-[11′,12-$^3H_2$]farnesol (**5**) and (*E*,*E*)-[2-^{14}C]farnesol (**6**) (both from earlier syntheses[4,2]) yielding a sample in which 67.6% of the ^{14}C label is located at C-2 and 32.4% at C-4 of (**7**), ^{3}H/^{14}C = 3.13 : 1.

The sample (**7**) was injected neat under CO_2 narcosis between two abdominal segments into the fat body of 10 male *Lytta vesicatoria*, collected in Sicily. After 24 h the beetles were killed by deep-freezing and the cantharidin (**8**) was isolated and purified to constant radioactivity.[4] To localize the ^{3}H and ^{14}C labels in (**8**) a new degradation procedure was designed for cantharidin, as shown in Scheme 2.

Cantharidin (**8**) was converted, *via* a dibromo-derivative,‡ into the crystalline diene (**9**), which was isolated, g.l.c. pure,



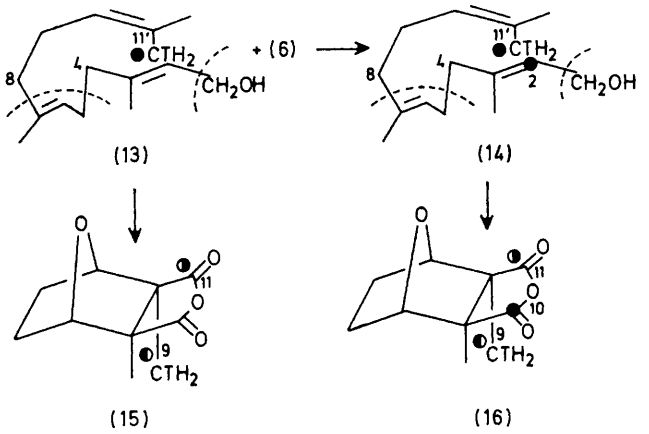
Scheme 2. *Conditions:* i, 33% HBr–AcOH, sealed tube, 170 °C, 24 h, LiBr–Li_2CO_3–*N*,*N*-dimethylformamide, reflux, 1.5 h; ii, benzene, sealed tube, 180 °C, 17 h.

in 57% yield. When heated in a sealed tube in the presence of the dienophile (**10**), (**9**) underwent a Diels–Alder/retro-Diels–Alder reaction resulting in ^{14}C-labelled dimethyl phthalate (**11**) and doubly labelled dimethylmaleic anhydride (**12**), both easily separated on t.l.c. to give g.l.c.-pure compounds in 97% yield. To complete the picture (**8**) was oxidised according to a modified Kuhn–Roth procedure[4] giving acetic acid labelled only with ^{3}H, as shown in Table 1.

Although for reasons of symmetry these degradations of cantharidin do not allow the detection of each single ^{14}C-label in (**8**), the figures in Table 1 conclusively prove the specific incorporation of C-4 of farnesol into cantharidin, since the ratio [4-^{14}C]/[2-^{14}C] in (**7**) is retained in the corresponding

† Geranyl bromide was condensed with diethyl [2-^{14}C]malonate; subsequent saponification, decarboxylation, and methylation resulted in (*E*)-[3-^{14}C]geranyl acetone which was converted into (**4**) according to ref. 4. Satisfactory spectroscopic data were obtained for all products from the syntheses of (**4**) and (**13**) and the degradation of (**8**). Whenever possible a g.l.c. identification (capillary column XE60[5]) was carried out with authentic samples. The separation of the (*E*,*E*)-isomers was accomplished by chromatography on 5% $AgNO_3$–silica gel. The purity of the synthetic samples was checked by radio-t.l.c.-scanning and g.l.c. to be ≥97%.

‡ The synthesis of the dibromo-derivative of cantharidin was optimised up to the best fitting microanalysis for Br. Because purification proved to be material consuming and irrelevant with respect to the result the degradation was carried out using the mixture of diastereoisomers.



Scheme 3

C-atoms of (**8**) with reference to the intramolecular ^3H standard.

In addition to the very unusual extrusion of the C-5 to C-7′ fragment of farnesol during the conversion into cantharidin the biosynthesis of (**3**), as depicted in Scheme 1 from [2-^{14}C]-mevalonate, exhibits a further aspect which is known from iridoid and related alkaloid biosyntheses,[6] *i.e.* that the application of [2-^{14}C]mevalonate to the biological system results in a randomisation of the label between the two C-atoms of the natural product which derive from the methyl groups of DMAPP (dimethylallylpyrophosphate). This randomisation might happen during the conversion B (Scheme 1)§ of farnesol (**2**) into cantharidin (**3**).

To investigate this possibility, the synthesis of (*E,E*)-[11′-^3H,^{14}C]farnesol (**13**) was carried out according to a previously described procedure.¶ Compound (**13**) was obtained in 11% yield from [^3H,^{14}C]methyl iodide ([^3H]- and [^{14}C]-methyl iodide obtained from Amersham, U.K.; purity 98%) displaying specific activities for ^3H of 17.5 mCi/mmol and for ^{14}C of 0.83 mCi/mmol, $^3H/^{14}C$: 21.1 : 1. For the two experiments described in Scheme 3, (**13**) was divided and one part was mixed with (*E,E*)-[2-^{14}C]farnesol (**6**) (from an earlier synthesis[4,2]) to give the multiply labelled sample (**14**) in which 56.0% of the ^{14}C-label is located at C-2 and 44.0% at C-11′, $^3H/^{14}C$: 9.29 : 1. Compounds (**13**) and (**14**) were injected neat into 25 and 29 male *Lytta vesicatoria*, respectively. In both experiments the beetles were killed after four days by deep-freezing and the cantharidin was isolated without dilution to establish for the first time an incorporation of 0.6% from the precursor farnesol. The cantharidin samples (**15**) and (**16**) were then diluted and degraded by modified Kuhn–Roth and Schmidt procedures.[4] The results are shown in Tables 2 and 3.

It is evident from the figures in Table 2 that randomisation takes place during the conversion of farnesol into cantharidin for the $^3H/^{14}C$ ratio drops down from 21.1 to 10.0 corresponding to 47% ^3H-retention. The argument is supported by the values for doubly labelled Kuhn–Roth acetic acid which exhibits the same $^3H/^{14}C$ ratio as the precursor (**13**) and half the molar ^{14}C-activity of the cantharidin (**15**).

§ The application of (*E,E,E*)-[11′-^3H,^{14}C]-12-hydroxyfarnesol and the corresponding dialdehyde unexpectedly failed to produce specifically labelled cantharidin, owing, probably, to penetration difficulties (ref. 7). The question is open whether randomisation also occurs during the biosynthetic sequence A (Scheme 1), ref. 8.

¶ The crucial steps in the synthesis of (**13**) are identical to those described in ref. 8 except the fact that for (**13**) careful attention was devoted to a clean separation of the (*E,E*)-isomer.† The starting material was the aldehyde obtained by cleavage of the terminal double bond of (*E,E*)-farnesyl benzyl ether.

Table 2. Degradation of [^3H,^{14}C]cantharidin (**15**) isolated from male *Lytta vesicatoria* after injection of (**13**).

	$^3H/^{14}C$	^3H rel.%	^{14}C rel.%
(**13**)	21.1 : 1	100 at C-11′	100 at C-11′
(**15**)[a]	10.0 : 1	100	100
$^{14}CTH_2CO_2H$[b]	20.0 : 1	96.7	48.1
$Ba^{14}CO_3$[c]	0	0	48.9

[a] Specific activity of (**15**): $^3H = 2.27 \times 10^7$ d.p.m./mmol, $^{14}C = 2.27 \times 10^6$ d.p.m./mmol. [b] Sum of C-2 + C-3 + C-8 + C-9. [c] Sum of C-10 + C-11.

Table 3. Degradation of [^3H,^{14}C]cantharidin (**16**) isolated from male *Lytta vesicatoria* after injection of (**14**).

	$^3H/^{14}C$	^3H rel.%	^{14}C rel.%
(**14**)	9.29 : 1	100 at C-11′	44 at C-11′ / 56 at C-2
(**16**)[a]	4.47 : 1	100	100
$^{14}CTH_2CO_2H$[b]	20.6 : 1	96.5	20.9
$Ba^{14}CO_3$[c]	0	0	79.0

[a] Specific activity of (**16**): $^3H = 1.20 \times 10^7$ d.p.m./mmol, $^{14}C = 2.68 \times 10^6$ d.p.m./mmol. [b] Sum of C-2 + C-3 + C-8 + C-9. [c] Sum of C-10 + C-11.

The degradation results of cantharidin (**16**), shown in Table 3, unequivocally demonstrate that both ^{14}C-labels of (**14**) are incorporated into cantharidin with retention of their ratio in the precursor, thereby proving the specific incorporation of C-11′ and C-2 of (**14**) and supporting the idea that the farnesol fragments C-2 to C-4 and C-8 to C-12 do not depart from the surface of the enzyme which performs this unusual oxidative cyclisation leading to cantharidin.

We thank the Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung for generous financial support.

Received, 13th October 1982; Com. 1196

References

1 J. R. Sierra, W.-D. Woggon, and H. Schmid, *Experientia*, 1976, **32**, 142; Ch. Schlatter, E. E. Waldner, and H. Schmid, *ibid.*, 1968, **24**, 994; H. Günther, E. Ramstad, and H. G. Floss, *J. Pharm. Sci.*, 1969, **58**, 1274.
2 W.-D. Woggon, M. G. Peter, and H. Schmid, *Helv. Chim. Acta*, 1977, **60**, 2288.
3 M. G. Peter, H.-R. Waespe, W.-D. Woggon, and H. Schmid, *Helv. Chim. Acta*, 1977, **60**, 1262.
4 M. G. Peter, W.-D. Woggon, Ch. Schlatter, and H. Schmid, *Helv. Chim. Acta*, 1977, **60**, 844.
5 K. Grob, *Helv. Chim. Acta*, 1965, **48**, 1362; 1968, **51**, 718.
6 R. Guarnaccia and C. J. Coscia, *J. Am. Chem. Soc.*, 1971, **93**, 6320; C. J. Coscia, L. Botta, and R. Guarnaccia, *Arch. Biochem. Biophys.*, 1970, **136**, 498; F. McCapra, T. Money, A. I. Scott, and I. G. Wright, *Chem. Commun.*, 1965, 537; H. Goeggel and D. Arigoni, *ibid.*, p. 538; A. R. Battersby, R. T. Brown, R. S. Kapil, A. O. Plunkett, and J. B. Taylor, *ibid.*, 1966, 46; D. A. Yoewell and H. Schmid, *Experientia*, 1964, **20**, 250; J. E. S. Hüni, H. Hiltebrand, H. Schmid, D. Gröger, S. Johne, and K. Mothes, *ibid.*, 1966, **22**, 656; S. Escher, P. Loew, and D. Arigoni, *Chem. Commun.*, 1970, 823; A. R. Battersby, S. H. Brown, and T. G. Payne, *ibid.*, p. 827.
7 W.-D. Woggon, unpublished results.
8 W.-D. Woggon, F. Ruther, and H. Egli, *J. Chem. Soc., Chem. Commun.*, 1980, 706; W.-D. Woggon, unpublished experiments.