Enzymatic Analysis. All enzyme assays were performed at 29.6 °C in an SLM Instruments fluorescence spectrometer. Excitation and emission wavelengths were 313 and 390 nm, respectively (both with an 8-nm bandwidth). All buffers contained 50 mM Tris·HCl, 50 mM KCl, and 2.5 mM $MgCl_2$, pH 7.80. Stock solutions were prepared as follows: FMN, 700 μM in buffer; NADPH, 880 μM in buffer; L-glutamine, 200 mM in water; substrate and inhibitor, 1.0 mM in water; anthranilate synthase, 8.8 nkat/mL in 20 mM potassium phosphate buffer (pH 7.1) containing 0.5 mM EDTA and 0.1 mM 2-mercaptoethanol; and chorismate synthase, 3.9 nkat/mL in 50 mM Tris·HCl (pH 7.5) and 0.5 mM DTT in 50% glycerol (v/v). For use in the assay, the chorismate synthase solution was diluted 25-fold with the buffer described above.

Standard curves of anthranilate concentration vs. fluorescence intensity were prepared. A full-scale deflection (25.4 cm) usually represented 0.5–5 nmol of chorismate. For the assay of chorismate synthase, the reaction mixture contained the following: chorismate synthase, 3.14 pkat; anthranilate synthase, 440 pkat; FMN, 10 μM; NADPH, 44 μM; L-glutamine, 5 mM; Tris·HCl (pH 7.80), 50 mM; KCl, 50 mM; and $MgCl_2$, 2.5 mM. For inhibition studies, iso-EPSP was also included. This mixture was preincubated at 29.6 °C for 5 min, and the reaction was started by the addition of 50 μL of a substrate solution of appropriate concentration. The total volume of the reaction mixture was always 2.00 mL. The substrate concentrations were 1.0, 1.45, 2.5, 5, and 10 μM; inhibitor concentrations were 2.5, 5, 10, and 20 μM. The initial velocities were calculated manually from the plot of fluorescence intensity vs. time. Each point was the mean of two independent assays. The analysis of the data was carried out by using the HYPER program.[15] K_m(EPSP) and K_i(iso-EPSP) were calculated to be 2.7 and 8.7 μM, respectively.

Anthranilate synthase was assayed under the conditions of the chorismate synthase assay, except that chorismate synthase was omitted and the reaction was initiated by the addition of chorismate (15 μM).

Acknowledgment. We thank Manfred v. Afferden and Prof. N. Amrhein for their generous gifts of purified chorismate synthase and anthranilate synthase and for suggesting the sensitive coupled assay. Support for this work was provided by a grant from the National Institutes of Health (Grant GM-28965).

(15) Cleland, W. W. *Nature (London)* **1963**, *198*, 463–465.

Origin of Oxygen Atoms in Cantharidin Biosynthesized by Beetles

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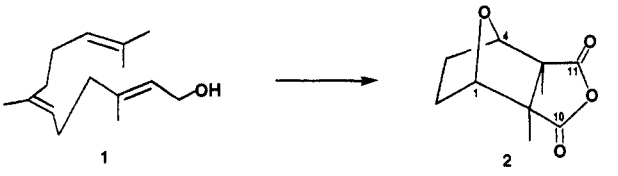
Abstract: Biosynthesis by blister beetles (Coleoptera:Meloidae) of the defensive substance cantharidin (**2**), an apparent monoterpene, proceeds by unprecedented degradation of farnesol (**1**), a sesquiterpenoid precursor. To obtain chemical insight into this transformation, we examined the origin of the four oxygen atoms in **2**. Labeling studies used adult male *Epicauta pestifera* that were exposed to either ^{18}O-enriched O_2 or H_2O. Analysis of the mass spectrometric data of the resulting **2** indicated that the tetrahydrofuranyl oxygen atom and two, but not three, of the anhydride oxygen atoms are derived from O_2, whereas the third anhydride oxygen atom comes from H_2O. Examination of maximally labeled **2** using mass spectrometry–mass spectrometry, which obviated complications owing to isotope dilution, revealed that the H_2O-derived oxygen atom is located in the anhydride ring in some molecules and in a carbonyl group in others, implicating intramolecular oxygen scrambling. Results indicate that O_2-derived incorporated oxygen atoms undergo no appreciable exchange with the medium. The possibility that **2** is a juvenile hormone metabolite is suggested.

Insects not only are the largest and most diverse group of organisms on earth but also are evolutionarily distant from vertebrates, plants, and bacteria. For these reasons, the study of insect metabolism promises to reveal novel biological chemistry. For example, use by some insects of homomevalonate to synthesize their juvenile hormone,[1] a homosesquiterpenoid, is unique among all types of organisms studied. An understanding of the unique metabolic features of insects may reveal insights regarding biological chemistry and could provide the foundation for new approaches for the control of pest insects.

Cantharidin (**2**), an apparent monoterpene that serves as a defensive substance in blister beetles,[2] is formed by an unprecedented degradation of the C_{15} farnesyl skeleton. Following early experiments,[3] an extensive series of ^{14}C radiolabeling studies[4] by Schmid and his collaborators demonstrated that the carbon atoms in **2** are derived from farnesol (**1**), which itself is derived from mevalonate in the normal manner. Intriguingly, the two terminal methyl groups of **1** are almost completely randomized during its conversion into **2**.[3,4d] Tritium labeling experiments demonstrated that all of the hydrogen atoms in **2**, with the exception of one attached to C-6, are derived from mevalonate.[3b,5] Unfortunately, knowledge of the origins of the carbon and hydrogen atoms in **2** fails to unveil chemical details regarding its biosynthesis beyond **1**.



1 2

Information regarding the origins of the oxygen atoms in **2**, in contrast, may provide chemical insight. Utilization of molecular oxygen implicates oxygen introduction during direct oxidation, such as that typical of C–H oxidation and of olefin epoxidation. Incorporation of oxygen from water is suggestive of introduction during alternative, nonoxidative processes, such as that typical of addition of water to an olefinic functionality and of nucleophilic displacement. Also, the extent of oxygen exchange with the medium and of intramolecular oxygen scrambling may be detected by ^{18}O studies, offering evidence about the involvement or absence

(1) (a) Schooley, D. A.; Judy, K. J.; Bergot, B. J.; Hall, M. S.; Siddall, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2921–2925. (b) Bergot, B. J.; Jamieson, G. C.; Ratcliff, M. A.; Schooley, D. A. *Science (Washington, D. C.)* **1980**, *210*, 336–338.

(2) Carrel, J. E.; Eisner, T. *Science (Washington, D. C.)* **1974**, *183*, 755–757.

(3) (a) Schlatter, C.; Waldner, E. E.; Schmid, H. *Experientia* **1968**, *24*, 994–995. (b) Guenther, H.; Ramstad, E.; Floss, H. G. *J. Pharm. Sci.* **1969**, *58*, 1274.

(4) (a) Peter, M. G.; Waespe, H.-R.; Woggon, W.-D.; Schmid, H. *Helv. Chim. Acta* **1977**, *60*, 1262–1272. (b) Woggon, W.-D.; Peter, M. G.; Schmid, H. *Helv. Chim. Acta* **1977**, *60*, 2288–2294. (c) Peter, M. G.; Woggon, W.-D.; Schmid, H. *Helv. Chim. Acta* **1977**, *60*, 2756–2762. (d) Woggon, W.-D.; Hauffe, S. A.; Schmid, H. *J. Chem. Soc., Chem. Commun.* **1983**, 272–274.

(5) Schlatter, C.; Duersteler-Meier, A. *Chimia* **1970**, *24*, 33.

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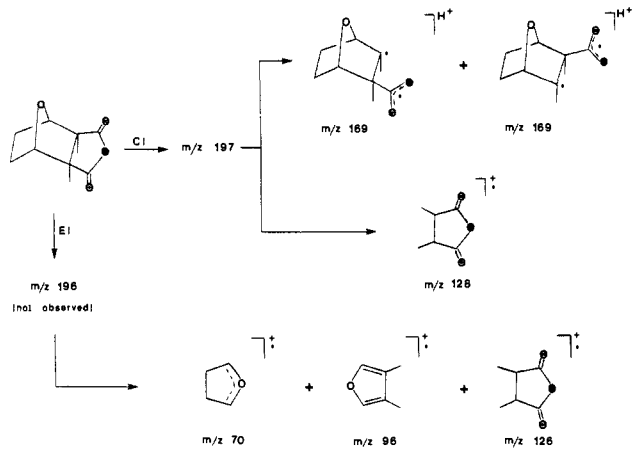
Scheme I



of intermediates having functionality together with conditions that permit such types of exchange.

Methods

Studies that employ ^{18}O labeling generally rely on mass spectrometry (MS) and ^{13}C NMR for detection and location of the label and therefore require much larger amounts of labeled materials and much higher incorporation levels than characteristically are needed for radioisotopic experiments, requirements that can be difficult to meet in studies of insect metabolism. A key to the success of our ^{18}O labeling experiments was the use of laboratory-reared blister beetles, *E. pestifera*, for which we knew the precise stage of development, age, and history of sexual activity, thus permitting administration of isotopically labeled materials under controlled conditions at an opportune time with respect to synthesis of **2**. We have found that in virginal *E. pestifera* adult males, rates of cantharidin biosynthesis 4–6 days after eclosion often exceed 0.25 mg/day.

For this study, it was important to learn, in addition to the extent of ^{18}O incorporation, the *biosynthetically determined* distribution of those oxygen atoms derived from $^{18}O_2$, information that could be learned directly by exclusive examination of the *maximally labeled* species. Thus, although NMR analysis of ^{18}O incorporation (by observation of its effects on ^{13}C signals)[6] has become common, its utility for our study was limited because the data were complicated by signals resulting from molecules of product that had experienced incomplete label incorporation. Also, our NMR data were unsatisfactory for quantitative interpretation, owing to the limited amount of material available and the typically low sensitivity for carbonyl carbon atoms.

Mass spectrometry permitted differentiation of molecules of **2** that were isotopically labeled to different extents. However, the fragmentation data were complicated by ions derived from incompletely labeled **2**; observed *fragment ions*, including isotopically shifted ions, could not be linked directly to derivation from the *maximally* labeled **2**. Our need to examine exclusively the maximally labeled material was met by using mass spectrometry–mass spectrometry (MS–MS), which provided direct information regarding the distribution of label derived from $^{18}O_2$ during biosynthesis of **2**.

Electron impact induced ionization (EI) and chemical ionization (CI) mass spectrometry methods provided complementary information regarding the isotopic composition of the four oxygen atoms of **2**. Scheme I shows the most informative ions produced by these two techniques.

The base peak at m/z 128 and the ions at m/z 70 and 96 in the EI mass spectrum provided valuable information about the oxygen isotopic composition in the anhydride and tetrahydrofuranyl portions of **2**. Abundances of potentially complicating ions at m/z 72, 98, 130, 132, and 134 were insignificant. However, EI did not provide a molecular ion in usable abundance, and therefore, the EI data did not permit direct observation of the extent of labeling in **2** or analysis of the isotopic distribution within the anhydride functionality.

The CI data were most useful regarding these two points. The protonated molecular ion, which was the base peak, permitted determination of the maximal extent of oxygen labeling in molecules of **2** recovered from the $^{18}O_2$ experiment. Measurement of the relative abundances of the ions that resulted from loss of CO (m/z 169 in unlabeled ions) permitted determination of the ^{18}O:^{16}O ratio in the carbonyl groups of the anhydride. Importantly, exact mass measurement showed that this m/z 169 fragment derives entirely from loss of CO; contribution from loss of ethylene could not be detected. Additionally, the formation of the m/z 128 ion by CI permitted corroboration of the data obtained from the m/z 128 ion that appeared as the base peak in the EI mass spectrum. Abundances in the CI mass spectrum of potentially complicating ions at m/z 130, 132, 134, 171, 173, and 175 were insignificant.

Experimental Section

Instrumentation. NMR data were obtained by using a Nicolet NT-300 wide-bore spectrometer; ^{13}C was observed at 75.45 MHz. Mass spectrometry data were obtained by using a Kratos MS-25 mass spectrometer with the DS-55 Kratos data system; samples were introduced by GC using a methyl silicone capillary column. Methane was used as the reagent gas for CIMS. MS–MS data were obtained by using a Kratos MS-50 mass spectrometer with a triple analyzer, sample introduction by solid probe, and isobutane as the reagent gas. The collision gas (He) was adjusted to attenuate the main beam by 50%; spectra were obtained by scanning the electrostatic analyzer on the second spectrometer. GC quantitation of **2** was accomplished using a Varian 3700 instrument with a 30-m DB-5 (J & W Scientific) capillary column and a Hewlett-Packard 3390A recorder/integrator.

Materials. The solvents and benzophenone were analytical reagent grade. The $^{18}O_2$ and $H_2{}^{18}O$ were obtained from the Monsanto Research Corporation MRC-Mound Laboratory, Miamisburg, OH; both were >95% ^{18}O enriched.

Blister Beetles. *E. pestifera*, collected in Boone County, MO, were used to obtain eggs that were cultured to adulthood in the laboratory by using Selander's method.[7] Adult male beetles were placed individually in plastic boxes 72–96 h after eclosion, kept at 24–26 °C under continuous illumination, and provided an artificial diet.[8] At the beginning of the incorporation experiments, all beetles were 100–120 h past eclosion and sexually inexperienced.

Extraction and Quantitative Analysis of Cantharidin. To minimize analytical complications resulting from other materials present in the biomatrix, four separate samples were prepared from each beetle used in our studies. At the end of an experiment, blood was obtained from each beetle,[2] which then was frozen and subsequently dissected into three portions: the third pair of male reproductive glands (where **2** is stored), the remainder of the reproductive system, and the remainder of the body. Previously described procedures[9] were used for extraction and subsequent quantitation of **2**.

$^{18}O_2$ Exposure. Five beetles were kept for 6 days at 25 °C in a closed, 4-L vessel with a supply of artificial diet and H_2O in an atmosphere initially consisting of 1:3 $^{18}O_2/N_2$. After 4 days, 395 mL of additional $^{18}O_2$ was added to displace an equivalent amount of existing atmosphere. After 2 additional days, the atmospheric oxygen was nearly exhausted and the exposure was terminated. The beetles were immediately bled,[2] frozen, and dissected into three portions as described above, yielding four samples from each beetle.

$H_2{}^{18}O$ Exposure. After intraabdominal injection of 20 μL of $H_2{}^{18}O$ into each of the five beetles, they were kept for 6 days at 25 °C in a closed vessel with a supply of artificial diet and H_2O. The atmosphere in the vessel was exchanged with air daily. Insects were bled and dissected in a manner identical with that used in the $^{18}O_2$ experiment.

Results and Discussion

Extent of Cantharidin Biosynthesis and ^{18}O Incorporation during Exposure to $^{18}O_2$. Table I shows the amount of **2** contained in each of the four samples from all five beetles exposed to $^{18}O_2$. The

(6) Hansen, P. E. In *Annual Reports On NMR Spectroscopy*; Webb, G. A., Ed.; Academic: New York, 1983; Vol. 15, pp 105–234.

(7) Adams, C. L.; Selander, R. B. *Bull. Am. Mus. Nat. Hist.* **1979**, *162*, 137–266.

(8) Chippendale, G. M.; Cassatt, K. In *Handbook of Insect Rearing*; Singh, P., Moore, R. F., Eds.; Elsevier: Amsterdam, 1985; Vol. 2, pp 257–263.

(9) Carrel, J. E.; Doom, J. P.; McCormick, J. P. *J. Chromatogr.* **1985**, *342*, 411–415.

Table I. Cantharidin Content of Blister Beetle Tissues[a]

beetle	blood	accessory glands	reproductive system	body	total
		After $^{18}O_2$ Exposure			
1	31	995	22	556	1604
2	113	751	54	686	1604
3	150	1314	38	439	1941
4	22	1128	1207	75	2432
5	192	429	30	570	1221
		After $H_2{}^{18}O$ Exposure			
6	10	2345	35	468	2858
7	NA[b]	1510	78	1260	2848
8	NA[b]	462	25	647	1134
9	35	1400	15	430	1880
10	172	1611	25	1108	2916

[a] Amounts in μg. [b] No blood obtained.

Table II. Relative Abundance Ratios for Selected Ions in Mass Spectra of Blood-Borne Cantharidin Formed during $^{18}O_2$ Exposure[a]

MS	ions	ratios
CI	197:199:201:203:205	13:31:39:16:1
CI	128:130:132:134	43:41:16:0
EI	128:130:132:134	38:44:17:1
EI	96:98	38:62
EI	70:72	29:71

[a] Data for beetle 1.

total amounts were typical for adult male *E. pestifera* approximately 10 days after eclosion, providing reassurance that cantharidin biosynthesis was normal during the experiment. Some variability in the distribution of **2** among the four types of samples for a given beetle presumably is normal, but a portion of the variability, particularly the strikingly different distribution shown for beetle 4, certainly resulted from technical difficulties associated with dissection.

The CIMS data showed extensive incorporation of ^{18}O into **2** in all samples examined by mass spectrometry: those derived from the blood, accessory glands, and remainder of the body from beetles 1, 2, and 3.[10] The relevant, key data from beetle 1 for blood-borne **2** were typical and are shown in Table II. Analysis of ^{18}O incorporations was best done on samples derived from the blood, because they uniformly proved to be least diluted with **2** synthesized prior to exposure to $^{18}O_2$.

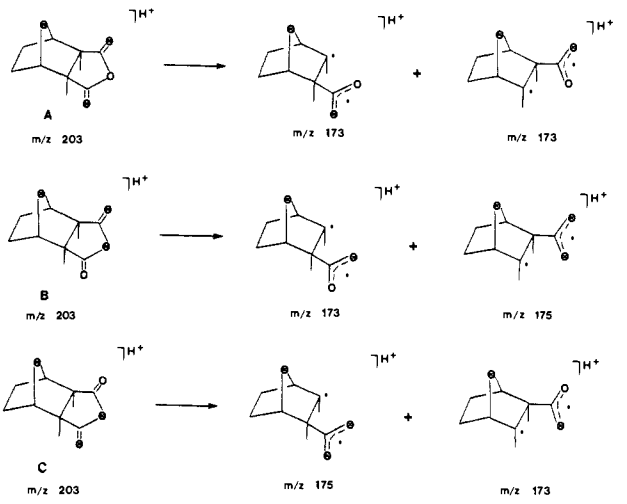
The ratios of the relative abundances (Table II) of the ions at *m/z* 197, 199, 201, 203, and 205 and at *m/z* 128, 130, 132, and 134 support the conclusion that three, but perhaps in no instance four, of the oxygen atoms in **2** are derived from O_2. The 199:201:203 ion ratios were almost exactly those (33.4:40.8:16.6) that would result from statistically determined selection of three oxygen atoms from a source having an ^{18}O enrichment of 55%.[11] If all four oxygen atoms in **2** were O_2 derived, this level of effective enrichment would have led to a statistically determined 9% abundance of the *m/z* 205 ion, a value far larger than that observed (ca 1%). Furthermore, the complete absence of the *m/z* 134 ion, which would result from isotopic shift of *m/z* 128 if all three anhydride oxygen atoms were labeled, provided sound evidence that the small relative abundance of the *m/z* 205 ion did not result from species that contained four ^{18}O atoms.

Labeling of Tetrahydrofuranyl Oxygen Atom by $^{18}O_2$. The ratios of relative abundances (Table II) of ions at *m/z* 96/98 and 70/72, assigned to structures that contain only the tetrahydrofuranyl oxygen atom (Scheme I), in the EI mass spectrum of blood-borne **2** clearly demonstrated ^{18}O incorporation into that atom; the extent of isotope incorporation into those ions in both cases is reasonably in line with an effective 55% ^{18}O enrichment.

(10) Samples from the reproductive system (minus the third pair of accessory glands) contained materials that eluted from the GC simultaneously with **2**, preventing reliable interpretation of the mass spectrometry data for these samples.

(11) For technical reasons associated with preserving viability of the beetles, reduction of the isotopic enrichment level by dilution with atmospheric O_2 was expected.

Scheme II



The ^{13}C NMR data of **2** in the material obtained by combining the accessory gland samples from beetles 1, 3, and 4 provided corroborating support for this conclusion. The signal from C-1 and C-4 typical of unlabeled material at 84.708 ppm was accompanied by a second signal at 84.679 ppm. The 0.029 ppm upfield shift of this carbon signal is reasonable[6] for [^{18}O]ether. The relative intensities of the unshifted and upfield signals were 2:3, in close agreement with the isotopic enrichment indicated by the mass spectrometric data.

Labeling of Anhydride Oxygen Atoms by $^{18}O_2$. ^{13}C NMR data of **2** from the accessory glands provided qualitative evidence that within the anhydride functionality ^{18}O appeared at both types of positions. The characteristic carbonyl carbon signal at 175.400 ppm was accompanied by higher field signals (shifts of −0.011, −0.020, and −0.038 ppm),[6,12] revealing the existence of three types of carbonyl carbons in addition to those devoid of label: those bearing ^{18}O (1) only in the ring, (2) only in the carbonyl group, and (3) in both positions.

The EI and CI mass spectrometric relative abundances (Table II) of the *m/z* 128 ion and its isotopically shifted companions revealed that some molecules contained two labeled oxygen atoms within the anhydride functionality. The absence in the CI mass spectrum of the *m/z* 134 ion demonstrated that no molecules contained three labeled oxygen atoms in the anhydride group. This agrees with the conclusions that (1) no molecules of **2** contained four $^{18}O_2$-derived oxygen atoms and (2) the tetrahydrofuranyl oxygen atom is derived from O_2.

The distribution of ^{18}O label within the anhydride functionality as it resulted solely from biosynthetic events was revealed by exclusive examination of the mass spectrometric behavior of the maximally labeled *m/z* 203 ion. MS–MS analysis of this ion showed that the *m/z* 128 ion normally observed in the CI mass spectrum of **2** was shifted entirely to *m/z* 132; no *m/z* 130 or 134 ions were observed. This confirmed the conclusions that in maximally labeled **2** two (but never three) of the anhydride oxygen atoms were labeled and that the tetrahydrofuranyl oxygen atom was completely labeled.

The *m/z* 203 ion, therefore, could comprise three different species, shown in Scheme II as A, B, and C, representing the three possible arrangements of two labeled and one unlabeled anhydride oxygen atoms. The arrangements of the label in species B and C are indistinguishable by mass spectrometry but biosynthetically are different.[13] Loss of carbon monoxide from A leads exclusively to the *m/z* 173 ion, regardless of whether its formation involves loss of C-10 or C-11. Loss of carbon monoxide from B or C results in loss of C-10 or C-11 with equal frequency, leading in the

(12) Comparison data for a lactone with a multiple labeling pattern: Simpson, T. J.; Stenzel, D. J.; Moore, R. N.; Trimble, L. A.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1984**, 1242–1243.

(13) The mirror-image halves of **2** are distinct in their biosynthetic origins.[4a]

Table III. Relative Abundances for M^+ Ions Derived from Authentic Cantharidin and That Formed during $H_2{}^{18}O$ Exposure[a]

sample	m/z 197	m/z 198	m/z 199
theoretical[b]	100	11.15	1.34
authentic	100	11.05	1.49
accessory glands	100	11.14	3.51
reproductive system	100	10.86	3.09
body	100	12.00	2.91
blood	100	10.86	1.60

[a] Data for beetle 6. [b] Calculated for $C_{10}H_{12}O_4$.

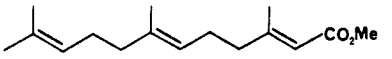
aggregate to formation of equal amounts of ions at m/z 173 and 175. When the total of the observed abundances of these two ions is set to be equal to 100%, the percent abundance of the m/z 175 ion then is equal to one-half of the amount of B + C, relative to the total amount of A + B + C.

The MS–MS spectrum of the m/z 203 ion showed that it produced ions at m/z 173 and 175 in a ratio of approximately 100:60. This corresponds to 37.5% of the m/z 175 ion, or 75% of species B and C, and, therefore, 25% of the symmetrically labeled A. It is clear that most of the anhydride *ring* oxygen atoms were labeled and that both carbonyl oxygen atoms were labeled to some extent, although these data do not permit determination of the relative amounts of B and C or indeed even whether only one exists.

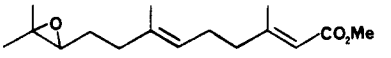
Extent of Cantharidin Biosynthesis and ^{18}O Incorporation during Exposure to $H_2{}^{18}O$. The inference that one oxygen atom of **2** is derived from water was supported by a complementary experiment: Five beetles were injected with $H_2{}^{18}O$ and kept for 6 days under conditions similar to those used for $^{18}O_2$ exposure. Workup provided four samples from each beetle, all of which contained typical total amounts of **2**, as shown in Table I.

The CIMS data for **2** contained in the samples from the accessory glands, reproductive system, and body showed small but experimentally reliable[14] enhancement of relative abundances of the m/z 199 ion; the data for beetle 6 were typical and are given in Table III. The low levels of ^{18}O incorporation presumably reflect expected extensive dilution of the $H_2{}^{18}O$ by $H_2{}^{16}O$ present in the beetles and in their environment during the experiment. A more convincing level of incorporation of label from $H_2{}^{18}O$ will require experimental methodology that ensures substantially less dilution by $H_2{}^{16}O$.

Biosynthetic Implications. The demonstration that the tetrahydrofuranyl oxygen atom is introduced by an oxidative process that utilizes O_2 is consistent with its introduction by epoxidation of an olefin as well as by C–H oxidation. Introduction either by hydration of an olefin or by some process in which water acts as a nucleophile is excluded. This result and the report[15] that methyl farnesoate (**3**) is incorporated into **2** at levels approaching those of incorporation of **1** raise the possibility that **2** and the juvenile hormone of these insects share a portion of the same biosynthetic pathway beyond **1**. The genuine juvenile hormone of blister beetles is presumed to be methyl 10,11-epoxyfarnesoate (**4**),[1,16] biosynthesized by direct epoxidation of **3** or the corresponding acid.[17] It therefore is biosynthetically plausible that the tetrahydrofuranyl oxygen is introduced initially by epoxidation of the terminal double bond of an acyclic C_{15} precursor. Subsequent epoxide opening at some stage during biosynthesis of **2** could be linked to chemistry at C-11 that involves either carbon–carbon bond formation or adjacent methyl group functionalization.



3



4

Derivation of two oxygen atoms of the anhydride functionality from molecular oxygen implicates the involvement of direct oxygenation processes. Whatever they may be, the overall process of anhydride formation evidently involves functionality and conditions that permit significant intramolecular oxygen scrambling, since all three anhydride oxygen atoms of **2** ultimately are derived at least partially from O_2.

The ratios of $[^{18}O_1]$-, $[^{18}O_2]$-, and $[^{18}O_3]$cantharidin biosynthesized during exposure to $^{18}O_2$, reflected by the CI mass spectrometric data obtained from blood-borne **2**, provided evidence that *subsequent* to incorporation of oxygen from O_2 there is little or no exchange of that oxygen with that in the medium. Exchange of $^{18}O_2$-derived oxygen with the medium would have increased, at the expense of maximally labeled material, the relative abundances of molecules of **2** having only two or one labeled oxygen atoms. However, the relative abundances (Table II) of the m/z 199, 201, and 203 ions closely matched those that would result from statistically determined selection from O_2 having a uniform ^{18}O enrichment (55%, vide supra).

These results provide chemical clues that must be accounted for by any proposed pathway for transformation of farnesol into cantharidin, which we suggest may be a metabolite of the putative natural juvenile hormone (**4**) of blister beetles. Experiments currently are under way to test this hypothesis.

Acknowledgment. The National Institutes of Health provided financial support (Grants AM31186-03 and BRSG RR07053). The National Science Foundation provided funds for the NMR (Grant PCM-8115599 to the UMC NMR Center) and MS facilities (Grant PCM-8117116 to the UMC Chromatography–Mass Spectrometry Laboratory and CHE-8211164 to the Midwest Center for Mass Spectrometry, an NSF regional instrumentation facility). The expertise of the personnel at these three facilities is gratefully acknowledged.

(14) Relative abundance values (Table III) for m/z 197, 198, and 199 were obtained by multiple mass spectrometry scans and computer averaging.

(15) Sierra, J. R.; Woggon, W.-D.; Schmid, H. *Experientia* **1976**, *32*, 142–144.

(16) Except for Lepidoptera, higher homologues of **3** rarely are natural juvenile hormones for insects. Schooley, D. A. In *Analytical Biochemistry of Insects*; Turner, R. B., Ed.; Elsevier: Amsterdam, 1977; pp 241–287.

(17) Kramer, S. J.; Law, J. H. *Acc. Chem. Res.* **1980**, *13*, 297–303.