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Biosynthesis of Methacrylic Acid and Isobutyric acids in a Carabid Beetle, *Scarites subterraneus*[1]

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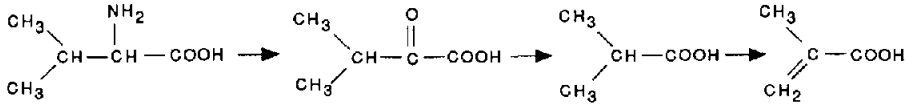
Abstract: *D_8-L-Valine is incorporated with high efficiency into methacrylic and isobutyric acids in the pygidial defensive glands of a carabid beetle, Scarites subterraneus.*

While carabid beetles are well known to produce a wide variety of defensive compounds,[2,3] the biosynthetic pathways by which these substances are produced have not been studied extensively. Methacrylic acid (**4**), an important constituent in the secretions of many carabids, was the subject of one early biosynthetic investigation by Benn *et al.*,[4] who observed the low incorporation (<0.03%) of radioactivity from 4-^{14}C-L-valine into **4** in *Carabus taedatus*. Although Wheeler *et al.* had suggested previously that **4** is derived from L-valine (**1**) via isobutyric acid (**3**) (Scheme 1),[5] none of the postulated intermediates has been experimentally demonstrated in this biosynthesis.



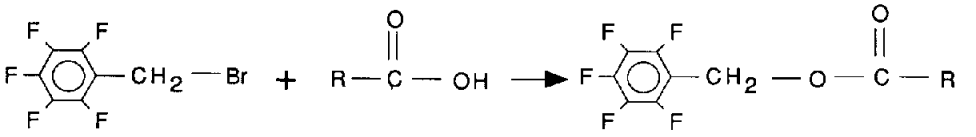
1 **2** **3** **4**

Scheme 1

We have recently found that the North American carabid beetle *Scarites subterraneus* produces a defensive secretion containing both isobutyric acid (**3**) and methacrylic acid (**4**), along with isocrotonic, crotonic, angelic, and tiglic acids.[6] The co-occurrence of **3** and **4** provided a good opportunity to examine the pathway to these four-carbon branched-chain acids in more detail. Using D_8-L-valine, we have now demonstrated that this amino acid can serve as an efficient precursor to both isobutyric and methacrylic acids.

Approximately 20% of the labeled valine administered to the beetle was incorporated into methacrylic acid in this experiment.

A solution of D_8-L-valine [$(CD_3)_2CDCD(NH_2)(COOH)$, Cambridge Isotope Laboratories; 5mg/100 µL saline)] was prepared and 5 µL aliquots were injected into three adult beetles. Control beetles received only the solvent (saline). After two days the beetles were frozen and the defensive glands were excised. The excised glands were crushed separately in hexane (100 µL). Pentafluorobenzyl (PFB) esters of the acids present in the extracts were made by adding pentafluorobenzyl bromide (Aldrich, 10 µL) and triethylamine (Aldrich, 10 µL)[7]. After 24 hr, 30 µL of water was added and the PFB esters were extracted into hexane (25 µL). The hexane layers were examined directly by GC[8] and GC-MS[9].



5 R ; $(CH_3)_2CH$
6 R ; $CH_2=C(CH_3)$
7 R ; $(CD_3)_2CD$
8 R ; $CD_2=C(CD_3)$

A gas chromatogram of the PFB esters obtained from a glandular extract of a deuteriovaline-injected beetle is shown in Figure 1. Two new peaks (marked with stars in Figure 1) eluted immediately before those of the PFB esters of isobutyric (**5**) and methacrylic acid (**6**). It is known that deuterated compounds usually elute prior to those of the corresponding non-labeled compounds,[10,11] and we assumed those new compounds to be **7** and **8**, respectively. Chromatograms obtained from extracts of control beetles did not contain either of these peaks.

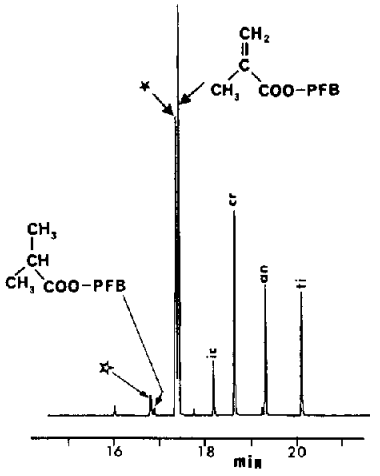


Figure 1. A gas chromatogram of PFB esters of acids obtained from deuteriovaline-injected beetles. Column DB-1. ic = isocrotonic acid, cr = crotonic acid, an = angelic acid, ti = tiglic acid

The identity of all GC peaks was established by obtaining the mass spectra of the corresponding compounds by GC-MS.[9] The *m/z* value of the molecular ion of the pentafluorobenzyl derivative of the perdeuterioisobutyric acid (**7**) obtained from deuteriovaline-injected beetles was seven mass units higher than that of isobutyric acid PFB ester (Figure 2), as required for the loss of the deuterium atom attached to the chiral center of valine.

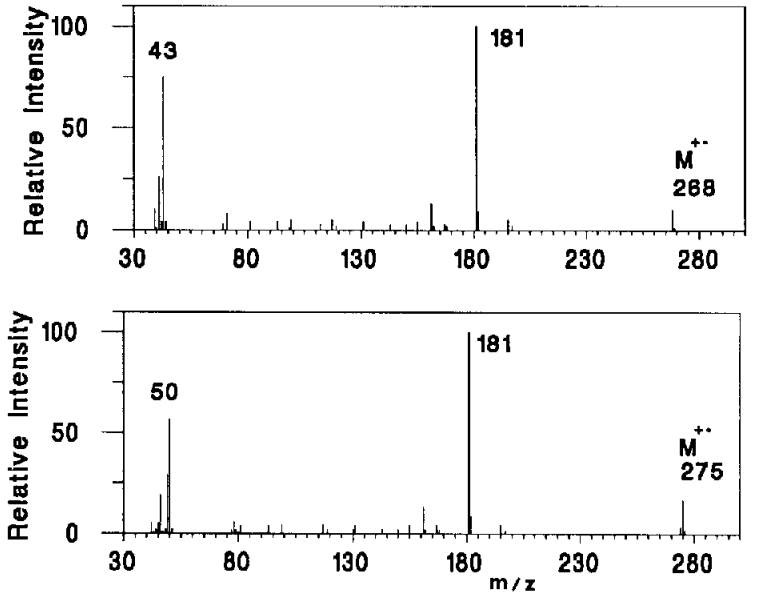


Figure 2. Mass spectra of PFB esters of **5** (A) and **7** (B).

From the mass spectra of PFB esters of perdeuteriomethacrylic acid (**8**) and methacrylic acid (**6**), it was evident that five deuterium atoms were retained in the labeled methacrylic acid (Figure 3). The mass spectral data also indicates that the peaks at *m/z* 41 and 69 represents $C_3H_5^+$ and $CH_2{=}C(CH_3){-}CO^+$ respectively; the corresponding perdeuterated analogs show corresponding *m/z* values at 46 and 74 (Figure 3). The formation of prominent ions at *m/z* 221 and 226 requires a rearrangement leading to a loss of a benzylic hydrogen atom along with the elements of carbon dioxide [$^{\bullet}CO_2H$]. While the mechanism for this unanticipated rearrangement is obscure, it must require a double bond in the acid moiety, since pentafluorobenzyl isobutyrate yields no analogous fragments.

It is interesting to note that the ratio of deuterium-labeled to unlabeled isobutyric acid is much higher than the corresponding ratio of methacrylic acids (Fig. 1). If both of these products stem from the same L-valine pool, how can this observation be rationalized? We suggest that a primary kinetic isotope effect operates during the dehydrogenation of isobutyric to methacrylic acid, resulting in the selective depletion of **3** to give unlabeled **4**. The consequent relative deuterium enrichment in the residual isobutyric acid and deuterium dilution in the methacrylic acid fraction is exactly what would be anticipated if the saturated acid were the precursor of its unsaturated analog, as suggested over two decades ago.[5]

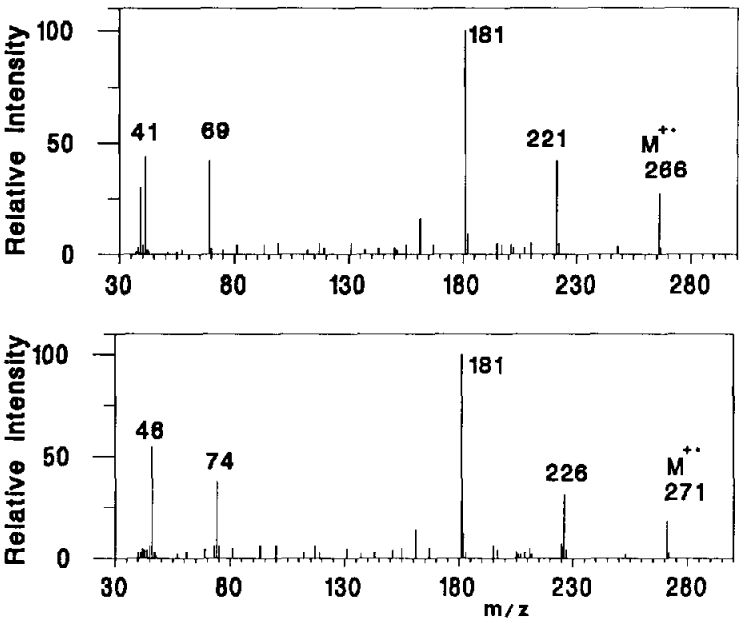


Figure 3. Mass spectra of PFB esters of **6** (A) and **8** (B).

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REFERENCES AND NOTES

1. Paper no. 105 of the series "Defense Mechanisms of Arthropods".
2. Blum, M.S. *Chemical Defenses in Arthropods*; Academic Press: New York. 1981.
3. Dettner, K. *Ann. Rev. Entomol.* **1987**, 32, 17-48.
4. Benn, M.H.; Lencucha, A.; Maxie, S.; Telang, S.A. *J. Insect Physiol.* **1973**, 19, 2173-2176.
5. Wheeler, J.W.; Chung, R.H.; Oh, S.K.; Benfield, E.F.; Neff, S.E. *Ann. Entomol. Soc. Am.* **1970**, 63, 469-471.
6. Attygalle, A.B.; Meinwald, J.; Eisner, T. in preparation
7. Attygalle, A.B.; Morgan, E.D. *Anal. Chem.* **1986**, 58, 3054-3058.
8. Hewlett-Packard (HP) 5890; DB-1 coated 25-m x 0.22-mm fused-silica capillary column; 30 °C for 4 min, 8°/min to 260 °C.
9. HP 5890 GC coupled to an HP 5970 mass selective detector; DB-wax coated 30-m x 0.22-mm capillary column; 60 °C for 4 min, 10°/min 180 °C; 70 eV EI spectra were recorded at 2 sec/scan.
10. Löfstedt, C.; Elmfors, A.; Sjögren, M.; Wijk, E. *Experientia* **1986**, 42, 1059-1061.
11. Bestmann, H.J.; Herrig, M.; Attygalle, A.B.; Hupe, M. *Experientia* **1989**, 45, 778-781.

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