Phytochemistry, 1968, Vol. 7, pp. 1027 to 1029. Pergamon Press. Printed in England

ADDENDUM. SPECTROSCOPIC CHARACTERIZATION OF TWO NEW AMINO ACIDS FROM *AESCULUS CALIFORNICA**

D. S. Millington and R. C. Sheppard

The Robert Robinson Laboratories, Liverpool University, Oxford Street, Liverpool 7

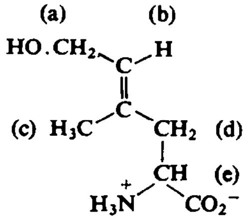
(*Received* 15 *November* 1967)

The nuclear magnetic resonance (NMR) and mass spectra of the novel amino acids (IV) and (V)† isolated from *Aesculus californica* by Fowden and Smith (preceding paper) have been determined. These spectra provide very strong evidence for the formulation of these amino acids as 2-amino-6-hydroxy-4-methylhex-4-enoic acid (IV) and 2-amino-3-(methylenecyclopropyl)butyric acid (β-methyl-β(methylenecyclopropyl)alanine) (V).

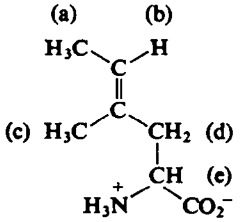
The NMR spectra were determined at 100 Mc/sec in deuterium oxide solution using 3-(trimethylsilyl)propan-1-sulphonic acid sodium salt as internal reference ($\tau = 10$). The mass spectra were recorded on an A.E.I. MS9 spectrometer at 70 eV using a direct insert sample probe at 300°. The elemental compositions indicated in parentheses for certain ions were confirmed by high-resolution mass measurements.

2-*Amino-6-hydroxy-4-methylhex-4-enoic acid* (*IV*). The NMR spectrum is given in Table 1, together with that of the closely related 2-amino-4-methylhex-4-enoic acid (I). The assignment of bands indicated in the formulae is straightforward. In all cases the integrated intensity, multiplicity, and chemical shift were consistent with the environment of the assigned proton(s). In addition to the bands indicated in the table, a small, non-stoichiometric singlet methyl resonance was present at 8·13τ. In view of the isolation of the compound by chromatography in a solvent system containing acetic acid, it is likely that this

TABLE 1. NMR SPECTRA OF 2-AMINO-6-HYDROXY-4-METHYLHEX-4-ENOIC ACID (IV) AND 2-AMINO-4-METHYLHEX-4-ENOIC ACID (I)



(IV)



(I)

(a) 5·89τ (doublet, J = 7 c/sec)		(a) 8·43τ (doublet, J = 7 c/sec)	
(b) 4·48τ (triplet, J = 7 c/sec)		(b) 4·55τ (quartet, J = 7 c/sec)	
(c) 8·30τ (singlet)		(c) 8·38τ (singlet)	
(d) ~7·5τ (multiplet)	ABX pattern	(d) ~7·6τ (multiplet)	ABX pattern
(e) 6·20τ (quartet)		(e) 6·23τ (quartet)	

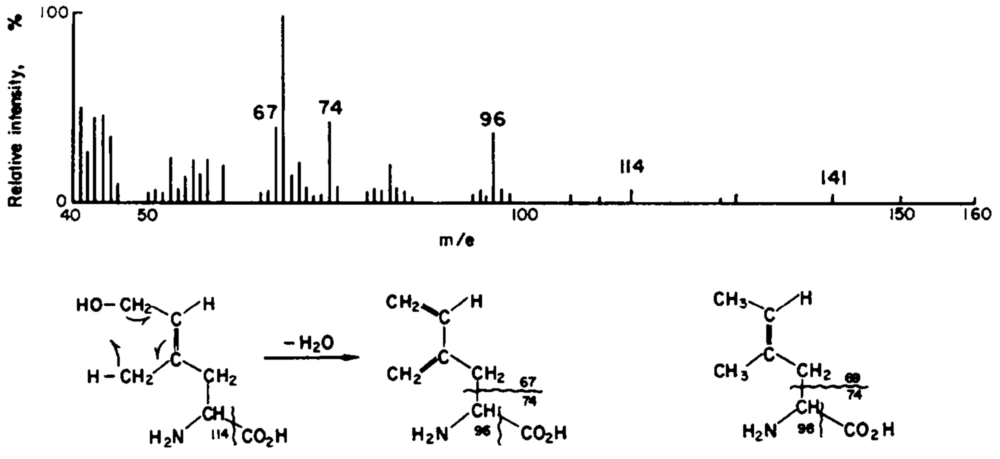
---

* Owing to a Publisher's error, this paper became separated from the article to which it is an Addendum. The article appears in *Phytochem.* 8, 809–819 (1968).

† Formulae are numbered as in L. Fowden and A. Smith, *Phytochem.* 7, 809 (1968).

peak is due either to residual traces of acetic acid, or to partial acetylation of the allylic hydroxyl group. Evidence for the presence of acetic acid was also obtained from the mass spectrum.

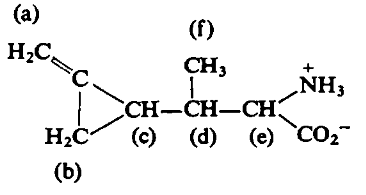
The mass spectrum (Fig. 1) is completely consistent with the assigned structure. The highest ion observed was at m/e 141 ($C_7H_{11}NO_2$), but this could not be the parent ion because, *inter alia*, the ion at m/e 114 ($C_6H_{12}NO$) has a higher hydrogen content. These and other major fragment ions at m/e 96, 74 ($C_2H_4NO_2$), and 67 (C_5H_7) are assigned as indicated by the bond fissions in Fig. 1(a) and (b), and follow the known fragmentations of α-amino acids.[1] They may be compared with the fragmentations of (I) indicated in Fig. 1(c).



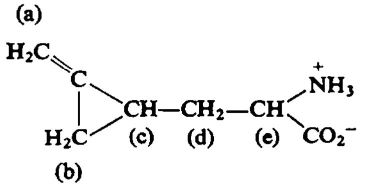
(a) M.W. 159 (b) m/e 141 (c) M.W. 143

FIG. 1. MASS SPECTRUM OF 2-AMINO-6-HYDROXY-4-METHYLHEX-4-ENOIC ACID (IV).

TABLE 2. NMR SPECTRA OF 2-AMINO-3-(METHYLENECYCLOPROPYL)BUTYRIC ACID (V) AND HYPOGLYCIN A (VI)



(V)



(VI)

(a) 4·60τ (multiplet)	(a) 4·59τ (multiplet)
(b) (c) 9·10, 8·67τ (multiplets)	(b) (c) 9·12, 8·6τ (multiplets)
(d) *ca.* 8·2τ (multiplet)	(d) 8·12τ (triplet)
(e) 6·43τ (doublet)	(e) 6·25τ (pair of triplets)*
(f) 8·91τ (doublet)	

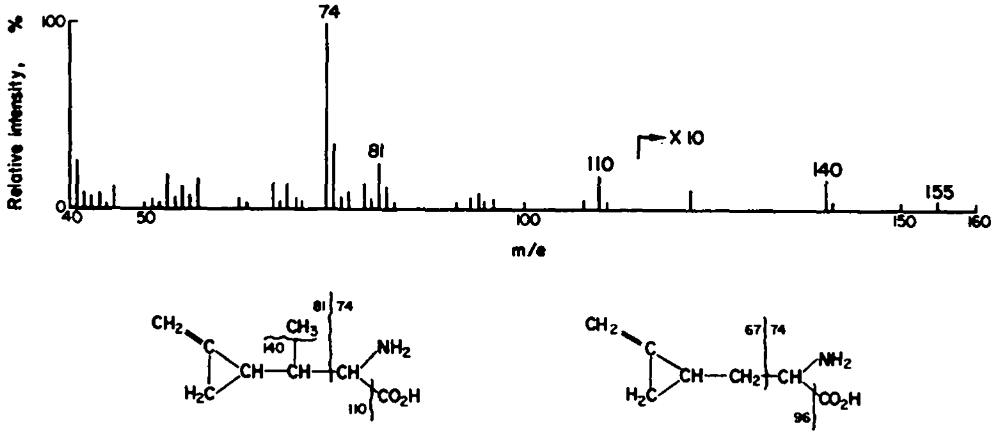
* The sample of (VI) was a synthetic mixture of diastereoisomers.

---

[1] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, p. 190, and Refs. there cited. Holden-Day, San Francisco (1964).

2-Amino-3-(methylenecyclopropyl)butyric acid (V). The NMR spectrum is given in Table 2, together with that of the closely related amino acid, hypoglycin A (VI). The total proton count could not be obtained accurately from the spectrum of (V) because of overlap with signals from the internal reference compound. However, a total of ten non-exchangeable protons was indicated and a corresponding H_{13} formula was subsequently confirmed by mass spectrometry (*infra*). The cyclopropyl and olefinic functional groups were indicated by bands at 9·1 and 4·60τ, and the spectrum was then assigned as indicated in the table. These assignments compare very well with those indicated for the very similar structure of hypoglycin A (VI).

In the mass spectrum (Fig. 2) a weak parent ion at m/e 155 ($C_8H_{13}NO_2$) gives rise to a fragment ion at m/e 140 ($C_7H_{10}NO_2$), indicating the presence of a methyl group. Other major fragment ions at m/e 110 ($C_7H_{12}N$), 81 (C_6H_9) and 74 ($C_2H_4NO_2$) may arise as indicated by the bond fissions in Fig. 2(a) and are consistent with the fragmentations of α-amino acids. The mass spectrum can be compared with that of hypoglycin A, whose fragmentations are summarized in Fig. 2(b).



(a) m/e 155 (b) M.W. 141

FIG. 2. MASS SPECTRUM OF 2-AMINO-3-(METHYLENECYCLOPROPYL)BUTYRIC ACID (V).