Tetrahedron, 1967, Vol. 23, pp. 2417 to 2419. Pergamon Press Ltd. Printed in Northern Ireland

# THE STRUCTURE OF A $C_{12}$-ACID FROM THE SEED OF *PHASEOLUS MULTIFLORUS*

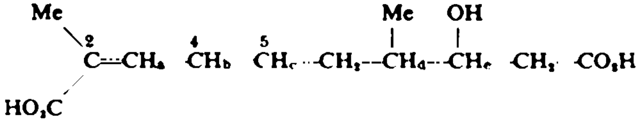
J. MacMillan* and P. J. Suter†

(*Received* 21 *October* 1966)

**Abstract**—Structure I has been assigned to an acid isolated in minute amount from the immature seed of *Phaseolus multiflorus*.

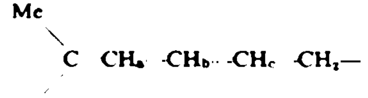
During the isolation[1,2] of gibberellins $A_1$, $A_5$, $A_6$ and $A_8$ from immature seed of *Phaseolus multiflorus*, we obtained an acid for which structure I is deduced from spectroscopic data.

The acid, available in small amount, was difficult to purify and definitive elemental analyses could not be obtained. The molecular formula, $C_{12}H_{18}O_5$, was derived from the mass spectrum of the acid and the liquid methyl ester. The presence of two carboxyl groups was shown by potentiometric titration and was supported by the



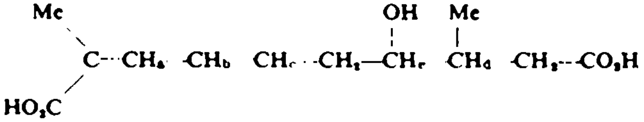
$$\underset{\mathrm{HO_2C}}{\overset{\mathrm{Me}}{\overset{2}{\mathrm{C}}}}=\mathrm{CH_a}-\overset{4}{\mathrm{CH_b}}-\overset{5}{\mathrm{CH_c}}-\mathrm{CH_2}-\overset{\mathrm{Me}}{\mathrm{CH_d}}-\overset{\mathrm{OH}}{\mathrm{CH_e}}-\mathrm{CH_2}-\mathrm{CO_2H}$$

I



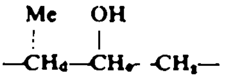
$$\underset{\mathrm{HO_2C}}{\overset{\mathrm{Me}}{\mathrm{C}}}\ \mathrm{CH_a}-\mathrm{CH_b}-\mathrm{CH_c}-\mathrm{CH_2}-$$

II



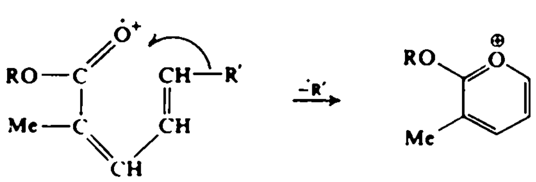
$$\underset{\mathrm{HO_2C}}{\overset{\mathrm{Me}}{\mathrm{C}}}-\mathrm{CH_a}-\mathrm{CH_b}-\mathrm{CH_c}-\mathrm{CH_2}-\overset{\mathrm{OH}}{\mathrm{CH_e}}-\overset{\mathrm{Me}}{\mathrm{CH_d}}-\mathrm{CH_2}-\mathrm{CO_2H}$$

III



$$-\overset{\mathrm{Me}}{\mathrm{CH_d}}-\overset{\mathrm{OH}}{\mathrm{CH_e}}-\mathrm{CH_2}-$$

IV



V VI

---

* Dept. of Organic Chemistry, The University, Bristol

† I.C.I. Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire

[1] J. MacMillan, J. C. Seaton and P. J. Suter, *Tetrahedron* **11**, 60 (1960).

[2] J. MacMillan, J. C. Seaton and P. J. Suter, *Tetrahedron* **18**, 349 (1962).

IR spectra of the acid and Me ester which also showed absorption due to an alcoholic OH group. Light absorption data indicated the presence of a 2,4-dienoic acid chromophore.

The PMR spectrum of the acid is shown in Fig. 1. The assignments in the olefinic region which are consistent with the partial structure II were confirmed by double resonance experiments. Irradiation of $H_a$ at 2·85 $\tau$ sharpened the vinylic Me signal at 8·15 $\tau$ and simplified the $H_b$ signal to a doublet with J, 14–15 c/s; also, irradiation at ca. 7·6 $\tau$ (allylic methylene protons) caused the collapse of $H_c$ to a doublet with J, 14–15 c/s. The assignment of protons (d) and (e) to the system IV was made (a) by irradiation of the methylene protons at ca. 7·7 $\tau$ which simplified $H_e$ at 6·16 $\tau$ to a doublet (J, 5 c/s) and (b) by irradiation of $H_d$ at ca. 8·25 $\tau$ which caused collapse of the methyl doublet at 9·1 $\tau$ and simplified $H_e$ to a double doublet (J, 4 and 8 c/s) resulting from coupling with the non-equivalent (chiral) methylene protons. Since the methylene protons of systems II and IV could not be distinguished by their chemical shift or by their multiplicity, it was not possible to decide between the arrangements I and III by PMR.

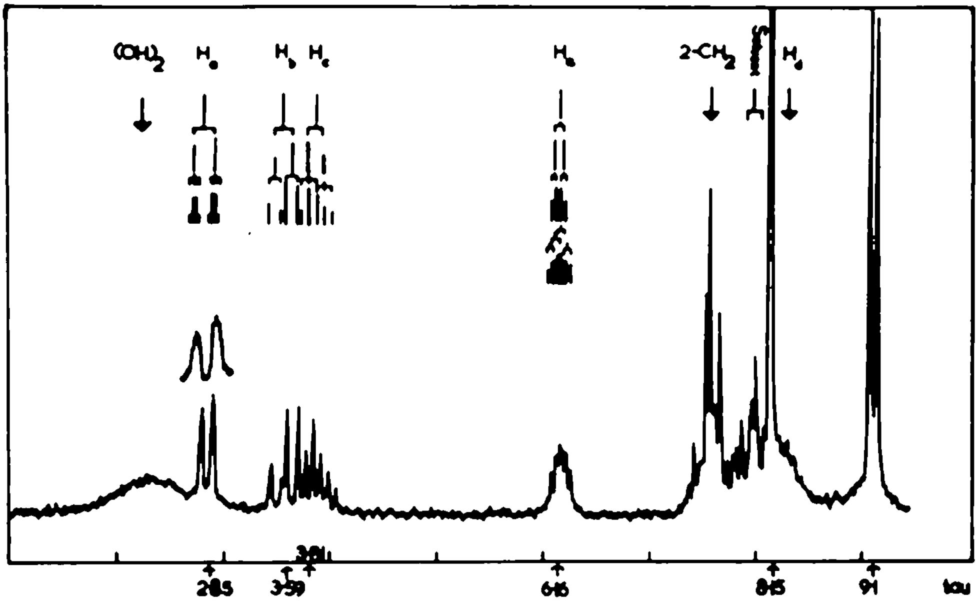


Fig. 1. The 100 Mc/s PMR spectrum of the acid I in $d_6$-acetone solution.

$$\begin{tikzcd}
 & m/e,\ 153\ (M{-}CH(OH){\cdot}CH_2CO_2H)^+ & \\
m/e,\ 182\ (M{-}MeCO_2H)^+ & M^+ (m/e,\ 242) \arrow[l, "m^*,\ 136{\cdot}9"'] \arrow[u, "m^*,\ 96{\cdot}7"'] \arrow[r, "m^*", "64{\cdot}5"'] \arrow[d] & m/e,\ 125\ (M{-}CH(Me){\cdot}CH(OH){\cdot}CH_2CO_2H)^+ \\
 & m/e,\ 111\ (M{-}CH_2{\cdot}CHMe{\cdot}CHOH{\cdot}CH_2CO_2H)^+ &
\end{tikzcd}$$

Fig. 2. Some fragmentations in the mass spectrum of the acid I.

The mass spectrum of the acid showed the sequential loss of three $H_2O$-fragments, each being followed by loss of CO; the appropriate metastable ions were observed for all 6 transitions. The fragmentations shown in Fig. 2 clearly establish the structure I. The ion of m/e, 111 which is the base peak probably arises by the transition V (R = H) → VI (R = H); this interpretation is supported by the absence of a peak of *m/e*, 111 in the spectrum of the dimethyl ester which had instead a strong peak at *m/e*, 125 attributable to the ion VI (R = Me). Such vinylic 5,6-cleavage to the stable ion VI is diagnostic of 2,4-dienoic acids.[3]

The available data do not permit a rigorous definition of stereochemistry, but the extinction coefficient of 27,540 at 263 mμ and the values of 1·2 and 14–15 c/s for the allylic and olefinic coupling constants point to a *trans*, *trans*-geometry.

## EXPERIMENTAL

IR spectra were obtained on a Grubb–Parsons S.3A spectrometer with a reflecting microscope attachment; the mass spectra on an A.E.I. M.S.9 spectrometer and the PMR spectra on a Varian H.A.100 spectrometer.

*Isolation of the acid* I. The extraction of immature seed (27·3 kg) of *Phaseolus multiflorus*, and chromatography of the derived crude acidic fraction (14·0 g) on columns A (charcoal-celite) and B (celite-silica gel), are described in Ref. 2. In that study fraction 17 (350 mg), eluted with water containing 50–51 % acetone from column A, was further chromatographed on column B from which gibberellin $A_4$ (55 mg) was eluted with Chf–AcOEt (4:1, 2 × 50 ml). We now report that further elution with Chf–AcOEt (4:1, 5 × 50 ml) gave a gum (126 mg) from which, after repeated crystallization from AcOEt–light petroleum (b.p. 60–80°), *the acid* I was obtained as needles m.p. 125–127°. (Found: C, 60·3; H, 7·3%; equiv. (potentiometric), 122; M(mass spectrum), 242·1154 $C_{12}H_{18}O_5$ requires: C, 59·5; H, 7·4%; equiv. (dibasic), 121; M, 242·1154). $\lambda_{max}^{EtOH}$ 263 mμ (ε, 27,540). $\nu_{max}^{nujol}$ 3425, 3330, *ca.* 2700 (broad), 1692, 1644 and 1606 cm⁻¹. Methylation with diazomethane gave the liquid *dimethyl ester* [Found: *M*(mass spectrum), 270·1459 $C_{14}H_{22}O_5$ requires *M*, 270·1467]; $\nu_{max}^{film}$ 3540, 1745, 1715, 1646 and 1620 cm⁻¹.

*Acknowledgements*—We thank Drs. G. R. Bedford, B. R. Webster and R. Binks for the NMR and mass spectra and for their helpful discussions.

[3] W. K. Rohwedder, A. F. Mabrouk and E. Selke, *J. Phys. Chem.* **69**, 1711 (1965).