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ISOLATION AND CHARACTERIZATION OF *N*-METHYL-L-SERINE FROM *DICHAPETALUM CYMOSUM*

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Abstract—A new amino acid present in gifblaar, *Dichapetalum cymosum*, was isolated and identified as *N*-methyl-L-serine. In very young leaves 0·80 per cent of the dry weight consisted of *N*-methyl-L-serine compared to 0·25 per cent in old leaves. The corresponding values for the flowers, rhizome tips, pericarp and seed were 1·3, 1·5, 0·8 and 0·1 per cent respectively.

DURING a systematic biochemical investigation of the fluoroacetate-containing plant gifblaar (*Dichapetalum cymosum*, Dichapetalaceae), which causes large losses of stock in the Northern Transvaal region of South Africa, two unidentified amino acids were encountered in the free amino acid fraction. One has already been shown to be *N*-methyl-L-alanine[1] and in this communication the isolation and characterization of the other amino acid (hereafter referred to as Z) is described.

By paper chromatographic analysis of different parts of gifblaar, it was shown that Z is always one of the dominant free amino acids. It has an R_f of 0·73 (standard deviation 0·02) in water-saturated phenol and an $R_{alanine}$ of 1·36 (standard deviation 0·06) in *n*-butanol/acetic acid/water (9:1:2·9, v/v).

Although young leaves have a higher concentration of Z than old leaves, it was decided to use old leaves for the extraction because they are more readily available and they also contain fewer other amino acids in a relatively high concentration. This extract after concentration and defatting, was fractionated on a Dowex 50-X4 cation exchange column (H^+-form). It was shown by paper chromatography that Z separated well from *N*-methylalanine, γ-aminobutyric acid, alanine and serine, but was contaminated with relatively small amounts of glutamic and aspartic acids; these contaminants were removed with the aid of a Dowex 1-X8 anion exchange column. After concentrating the fractions containing only Z to dryness, Z was crystallized from methanol/acetone. The recrystallized material analysed as follows: C,40·5; H,7·5; N,12·5. $C_4H_9NO_3$ required: C,40·3; H,7·6; N,11·8 per cent.

Although Z was not volatile, a mass spectrum was obtainable by working at a relatively high inlet temperature (190°) (Fig. 1). A small peak at *m/e* 119 indicated that Z could be provisionally formulated as $C_4H_9NO_3$. From the strong peak at *m/e* 74 it seemed as if Z was an unsubstituted alpha amino acid ($+NH_2{=}CH{-}COOH = 74$). The i.r. spectrum of Z indicated that the third oxygen atom was a hydroxyl and not an ether function. These deductions indicated that Z could be either threonine ($CH_3{-}CHOH{-}CH(NH_2)COOH$) or homoserine ($CH_2OH{-}CH_2{-}CH(NH_2)COOH$), but neither of these compounds give the peaks observed at *m/e* 42, 56, 88 and 89. Furthermore, they differ in R_f from Z.

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[1] J. N. ELOFF and N. GROBBELAAR *J. S. African Chem. Inst.* **20**, 190 (1967).

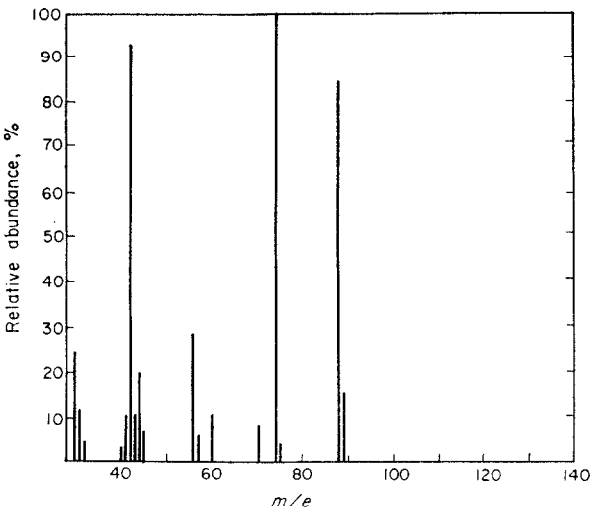

FIG. 1. MASS SPECTRUM OF Z.

Subsequently the ethyl ester of Z was prepared according to the method of Biemann and Vetter.[2] This compound gave a good mass spectrum at an inlet temperature of 80° (Fig. 2). A small peak at *m/e* 147 was ascribed to the molecular ion. The absence of a peak at *m/e* 102 ($+NH_2{=}CH{-}COOC_2H_5$) shows that Z is not an unsubstituted alpha amino acid (cf. Fig. 1). The fact that the base peaks in both Figs. 1 and 2 occur at *m/e* 74 (M^+-45(COOH), M^+-73($COOC_2H_5$)), indicates that Z is an alpha amino acid and is unsubstituted at the carboxyl group; therefore Z must be an *N*-substituted alpha amino acid. Only one of the six possible *N*-substituted alpha amino acids will give peaks at *m/e* 116, 30 and 89, namely *N*-methylserine. NMR analysis indicated the presence of a hydroxymethyl ($-CH_2OH$) group (doublet $\tau = 6{\cdot}05$, $J = 4{\cdot}3$) and a *N*-methyl ($\tau = 7{\cdot}25$) group thereby confirming the structure of Z as *N*-methylserine.

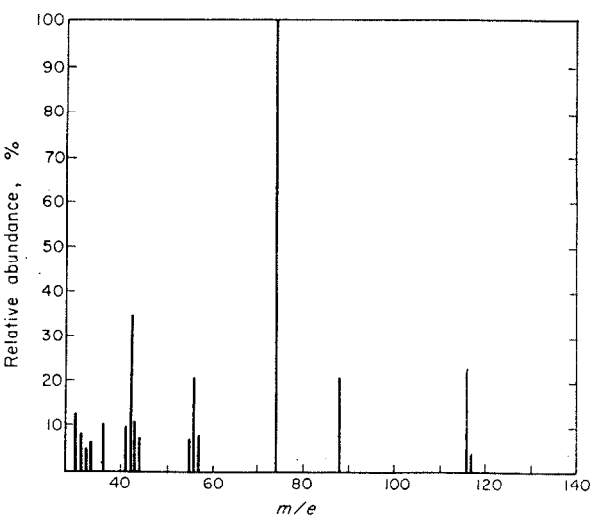

FIG. 2. MASS SPECTRUM OF THE ETHYL ESTER OF Z.

[2] K. BIEMANN and W. VETTER, *Biochem. Biophys. Res. Commun.* **2**, 93 (1960).

Additional evidence supporting the above conclusion is provided by the fact that Z gives the same rare, reddish brown colour with ninhydrin as sarcosine (*N*-methylglycine) and *N*-methylalanine. Furthermore the position of *N*-methylalanine relative to Z on paper chromatograms is the same as that of analogous pairs of amino acids and hydroxyl derivatives (e.g. alanine/serine, phenylalanine/tyrosine, etc.). The position of Z relative to serine is also the same as that of *N*-methylalanine to alanine and that of sarcosine to glycine.

As far as can be ascertained, *N*-methylserine has not previously been found in plants. For the unequivocal identification of Z, *N*-methyl-L-serine was synthesized according to the method of Quitt *et al.*[3] The i.r. spectra of Z and synthetic *N*-methyl-L-serine were superimposable. Furthermore, Z could not be separated by paper chromatography from the synthetic *N*-methyl-L-serine with any of the four solvents employed. Natural and synthetic material also produced the same reddish brown colour with ninhydrin. Although there was not enough of compound Z left for an accurate determination of the specific optical rotation, it was clear from comparing the results with the specific optical rotation as determined by Quitt *et al.*[3] that Z is the L-isomer.

Subsequently the concentration of Z was determined in different parts of Gifblaar (Table 1). It is interesting that the concentration of *N*-methylserine decreases upon ageing of the leaves. It is also known that the fluoroacetate content of Gifblaar is much lower in older than in young leaves.[4] The possibility that the metabolism of *N*-methylserine is linked with that of fluoroacetate was ruled out by finding that the phyllodes and seed of *Acacia georginae*, an Australian plant which contains fluoroacetate, lacked *N*-methylserine.

TABLE 1. *N*-METHYL-L-SERINE PRESENT IN DIFFERENT PARTS OF *Dichapetalum cymosum*

Plant part	% dry wt.
Very young leaves (< 2 in. long)	0·80
Immature leaves (> 2 in. long)	0·45
Mature leaves (not yet lignified)	0·38
Old leaves (fully lignified)	0·25
Rhizome tip	1·5
Blossoms	1·3
Seed	0·8
Pericarp	0·1

EXPERIMENTAL

Paper Chromatography

Two-dimensional chromatograms were developed by descent with water-saturated phenol, followed by a one-phase butan-1-ol–acetic acid–water mixture (90:10:29 v/v). Spots were revealed by spraying with 0·25% ninhydrin dissolved in 5% methanolic 2,4,6-collidine.

Extraction and Isolation

Fresh *Dichapetalum cymosum* leaves were macerated in an excess of 70% cold ethanol. After centrifugation the extract was concentrated and the aqueous residue extracted with $CHCl_3$. The defatted extract was fractionated on a Dowex 50-X4 cation exchange column (H^+-form) by eluting the column with HCl, gradually increasing from 1 N to 4 N.[5] Compound Z was freed from contaminating glutamic and aspartic acids by adsorption on a Dowex 1-X8 anion exchange column in the acetate form and eluting with 0·5 N HOAc.

[3] P. QUITT, H. HELLERBACH and J. VOGLER *Helv. Chim. Acta* **46**, 327 (1963).
[4] B. C. L. VON SYDOW, *Z. Pflanzenphysiol.*, in press.
[5] C. H. W. HIRS, S. MOORE and W. H. STEIN *J. Am. Chem. Soc.* **76**, 6063 (1954).

Quantitative Determination of N-*methylserine*

To determine the concentration of *N*-methylserine in different parts of *D. cymosum*, plant material was extracted four times in succession with a mixture of methanol, $CHCl_3$ and water (12:5:3).[6] The free amino acids were separated from the anionic and neutral components of the extract by passing the extract through a cation exchange column (Dowex 50-X4, H^+-form). After washing the column with water, the amino acids were displaced from the column with 0·1 N NH_4OH. Measured aliquots of the concentrated extracts and known amounts of the synthetic *N*-methylserine were applied to the same sheet of paper (Whatman No. 1) and developed with water-saturated phenol. After spraying with the ninhydrin/methanol/2,4,6-collidine mixture, the concentration of *N*-methylserine could be estimated ± 10 per cent.

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[6] R. L. BIELESKI and R. A. TURNER, *Anal. Biochem.* **17**, 278 (1966).