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2-C-METHYLALDOTETRONIC ACID, A NEW LACTONE-FORMING ACID PRESENT IN PLANTS

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Abstract—2-*C*-Methylaldotetronic acid (probably the *erythro* form) was found in considerable amounts in *Cannabis sativa, Cereus forbesii, C. peruvianus, Lophophora williamsii, Trichocereus santiaguensis, T. spachianus* and *T. strigosus*. In addition, the acid was present in minor amounts in another five species, all from the Cactaceae. In total, this new plant acid was detected in 12 of 19 investigated species.

INTRODUCTION

A further investigation of the lactone-forming acids in *Lophophora williamsii* [1] and *Cannabis sativa* [2] has led to the discovery of a 2-*C*-methylaldotetronic acid in both species. Fig. 1 shows the D-*erythro* isomer of the acid, in this paper called D-2-*C*-methylerythronic acid. The natural occurring acid may, however, have the L-configuration. To our knowledge this is the first report on any 2-*C*-methylaldotetronic acid in plants. We also include a preliminary screening for the presence of the acid in plants from five families.

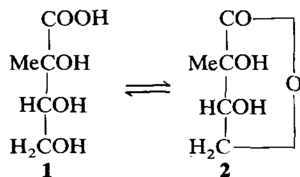

Fig. 1. The D-forms of 2-*C*-methylerythronic acid (**1**) and 2-*C*-methylerythronolactone (**2**).

RESULTS AND DISCUSSION

2-*C*-Methylaldotetronic acid was detected in 12 of 19 investigated species (Table 2). As will be discussed, the acid, most likely, occurs in the *erythro* form (Fig. 1). The MS spectra of the different derivatives of the acid (Table 1) correspond to those of a 2-*C*-methylaldotetronic acid; except for possible differences in the relative intensities of the peaks, diastereomeric acids give identical MS spectra [3, 4]. Hence, the configuration of the acid must be evaluated by other methods. Usually, the applied OV 17 column separates the TMSi derivatives of diastereomeric acids derived from carbohydrates [5]. The OV 225 column is known to separate diasteromeric alditol acetates; thus one would expect different retention times for the two 2-*C*-methyltetritols. Unfortunately, this could not be shown as only the *erythro* form was available as reference.

Chromatographed as its TMSi derivative, the new acid was eluted just before malic acid while its unlactonized form came slightly after malic acid (Table 1). This corresponds to the data given by Petersson for 2-*C*-methylerythronic acid [5]. The fact that three derivatives of the acid (Table 1) gave peaks with retention times identical with those of the corresponding derivatives of authentic 2-*C*-methylerythronic acid strongly indicated the presence of the *erythro* form of the new plant acid.

Acids may behave differently during ion-exchange chromatography and their TMSi derivatives may respond differently by the FID [6]. This makes quantitative determination of new acids difficult. However, the peak area ratios of the TMSi derivatives of 2-*C*-methylaldotetronic acid and citric acid give an idea of the amount of the new lactone-forming acid in the plants (Table 2). At least two of the species, *Cereus peruvianus* and *Opuntia ficus-indica* are known to exhibit crassulacean acid metabolism (CAM) [7]. Under certain conditions such plants are able to show diurnal fluctuation, mainly caused by malic acid, in total titratable acidity. Citric acid may participate, but its contribution to the diurnal variation is markedly less than that of malic acid [8]. Citric acid and malic acid were both found in all the plants examined, but because the level of citric acid is relatively constant during a day, this acid was selected as the internal standard for quantitative evaluation.

Recently, Paech *et al.* [9] postulated the formation

Table 1. GLC and GC–MS data on 2-*C*-methylerythronic acid

Compound	R_M*	70 eV *m/e* (rel. int.)
2-*C*-Methylerythronolactone diTMSi (MW 276)	0.93	276 [M$^+$] (9) 261 [M$^+$ − 15] (17) 233 [M$^+$ − 15 − 28] (15) 217 [M$^+$ − 59] (9) 189 (15) 177 (7) 147 (59) 133 (10) 116 (61) 101 (50) 73 (100)
2-*C*-Methylerythronic acid tetraTMSi (MW 438)	1.06	423 [M$^+$ − 15] (3) 393 [M$^+$ − 15 − 30] (3) 335 [M$^+$ − 103] (2) 321 [M$^+$ − 117] (2) 306 (24) 291 (3) 234 (15) 231 (5) 221 (2) 205 (45) 191 (6) 147 (42) 117 (39) 103 (8) 73 (100)
2-*C*-Methylerythritol tetraacetate (MW 304)	2.31	159 [M$^+$ − 145] (5) 129 (17) 117 (19) 103 (5) 87 (7) 75 (4) 43 (100)

* R_M = retention time relative to that of the corresponding derivative of malic acid.

of 2-*C*-methylaldotetronic acid 4-phosphate (*erythro* and *threo* forms) as β-elimination products formed by alkaline treatment of ribulose 1,5-bisphosphate (RuBP). The authors [9] discussed the possibility of the formation of such acids in chloroplasts. Experiments in our laboratory showed that 2-*C*-methylerythronic acid is not an artefact obtained from RuBP by the methods used and described here. Biochemically, it is interesting that the corresponding alditol, D-2-*C*-methylerythritol is found in nature [10, 11].

The result of screening (Table 2) indicates that 2-*C*-methylaldotetronic acid might be common in Cactaceae, as it was found in 11 of the 14 species investigated. The new acid was also found in a member of Moraceae, but not in members of three other families investigated. Although 19 species from 5 different families has been investigated for the presence of 2-*C*-methylaldotetronic acid, it is too early to say if this acid is of taxonomic interest. Its absence from some of the investigated species might be a question of abundance and the sensitivity of our method of detection.

Table 2. Occurrence of 2-*C*-methylaldotetronic acid (probably the *erythro* form) in plants

Species*	Amount of 2-*C*-methylaldotetronic acid relative to citric acid†
Equisetaceae	
Equisetum arvense L. [14]	—
Moraceae	
Cannabis sativa L.	0.2–1.0‡
Urticaceae	
Urtica dioica L. [15]	—
Crassulaceae	
Echeveria derenbergii Purp.	—
E. elegans Rose	—
Cactaceae	
Cereus forbesii Otto	0.3
C. peruvianus (L.) Mill.	1.1
C. peruvianus cv *monstruosus* (blue) DC.	trace
C. peruvianys cv *monstruosus* (green) DC.	—
C. winteraeneus hort.	—
Lophophora williamsii (Lem.) Coulter	0.4
Opuntia ficus-indica (L.) Mill.	—
Trichocereus pachanoi Br. et R.	trace
T. santiaguensis (Speg.) Backeb.	0.4
T. schickendantzii (A. Web) Br. et R.	trace
T. spachianus (Lem.) Ricc.	0.2
T. strigosus (Salm-Dyck) Br. et R.	0.6
T. terscheckii (Parm.) Br. et R.	trace
T. werdermannianus Backeb.	trace

* Plant families are listed according to Engler [13].

† Calculated from gas chromatograms: peak area ratios of the TMSi derivatives of the acids. — = not detected.

‡ Relative to citric acid, the new acid was present in different amount in the six varieties of *C. sativa* examined.

EXPERIMENTAL

All cacti, except *Opuntia ficus-indica*, were obtained through Löxa nursery, Baerum, Norway. *Echeveria elegans*, *E. derenbergii* and *Opuntia ficus-indica* were supplied by the Botanical Garden, University of Oslo, Norway. *Cannabis sativa* L. was grown from seeds of six different varieties kindly supplied by Prof. C. E. Turner, University of Mississippi, U.S.A., Prof. Z. Krejci, University of Olomouc, Czechoslovakia, and by the U.N. Narcotics Laboratory in Geneva, Switzerland. Identification of the plants was carried out by Prof. A. Nordal, and Dr. P. Sunding, the director of the Botancial Garden.

Isolation of acid mixtures. Fresh stems (cacti) and leaves (*Echeveria* sp.) were cut in pieces and boiled with 96% EtOH for 0.5 hr. After removal of EtOH by evapn, the residue was extracted twice with H_2O (100°) for 1 hr. The combined H_2O extracts were dialysed ×3 against distilled H_2O. The dialysable material was then treated with Dowex 50 W×8 (H^+), 20–50 mesh, followed by Dowex 1×8 ($HCOO^-$), 20–50 mesh. From the latter ion-exchanger, the acid fraction was eluted with 2 M HCOOH or 2 M HCl. The dried leaves of *C. sativa* were milled, extracted with H_2O and otherwise treated as described above. All plants were extracted between 9 am and noon.

Detection of 2-*C*-*methylaldotetronic acid.* The component acids of the acidic eluates were analysed by GLC and GC–MS. The TMSi derivatives of the acids and their corresponding sodium salts were separated on a column (300×0.2 cm) of OV 17 10% and the corresponding alditol acetates on a column (200×0.2 cm) of OV 225 3%. The chromatographic conditions were as earlier described [12], except the temp. was programmed at a rate of 2°/min for both columns. GC–MS was carried out with the apparatus described by Jellum *et al.* [16].

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