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A NEW LACTONE FROM WATER-STRESSED CHICKPEA

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Key Word Index—*Cicer arietinum*; Leguminosae; chickpea; water stress; solute accumulation; dihydroxylactone; 2-methyl-2,3,4-trihydroxybutanoic acid-1,4-lactone.

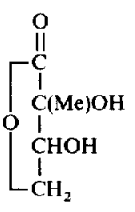
Abstract—The isolation of a new lactone, 2-methyl-2,3,4-trihydroxybutanoic acid-1,4-lactone, from leaves of water-stressed chickpea is described. Elucidation of the structure was by chemical and spectroscopic methods. The lactone could not be detected in the leaves of well-watered plants. Its occurrence in other species is confirmed.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of an important group of grain legumes grown in semi-arid tropical regions and which provides a valuable source of protein and oil for human and animal consumption. The results reported here describe the isolation of a new lactone from the leaves of water-stressed chickpea. This work formed part of an investigation relating phytochemical changes to physiological responses in legumes subjected to water deficits.

RESULTS AND DISCUSSION

The lactone was first detected by GLC after methanolic hydrogen chloride treatment of dried aqueous extracts of leaf tissue. The compound appeared as a single peak with a retention volume identical to that of isocitric acid, from which it was distinguished by TLC. The presence of a lactone structure was indicated by its behaviour in dilute alkali at room temperature which produced a substance which could be adsorbed on an anion exchange resin, but which, on acidification, reverted to a neutral form. The compound was also stable to hot dilute acid. These properties enabled the lactone to be considerably purified using ion exchange resins. TLC analysis of the finally purified compound showed a single component which reacted readily with reagents recommended for the detection of sugar esters and lactones [1]. The presence of hydroxyl groups and a saturated γ-lactone structure was indicated by the IR spectrum (ν 3400 and 1780 cm^{-1} respectively). The accurately determined MW together with the NMR data established the structure of the lactone to be 2-methyl-2,3,4-trihydroxybutanoic acid-1,4-lactone (1). The absolute configurations of C-2 and C-3 were not elucidated. However, from a consideration of the signs of optical rotation of *threo*- and *erythro*-pentuloses, the negative rotation of **1** may indicate a D-tetronic lactone. GLC of the TMSi derivative gave two peaks. The slower (minor) component may have resulted from under silylation, or else represented a second and possibly diastereoisomeric product. The former explanation is, however, indicated by the reduction of the lactone to the corresponding tetrol (**2**), whose TMSi ether gave a single peak in GLC.



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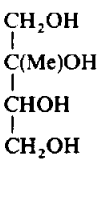

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Although lactone **1** has not previously been reported in higher plants, it is interesting to note that it is identical to one of the structures in a scheme postulated by Adelberg [2] to explain the formation of dihydroxy acid intermediates in the biosynthesis of valine in a mutant strain of *Neurospora*. The isolation of **1** in this work possibly offers support to Adelberg's hypothesis. Lactone **1** could not be detected in well-watered chickpea plants and hence accumulation of **1** may indicate a plant growth regulatory mechanism, involving feedback inhibition of the biosynthesis of valine, which comes into operation during water-stress situations. So far **1** has also been detected in leaves of the water-stressed legumes siratro (*Macroptilium atropurpureum*) and pigeon pea (*Cajanus cajans*), but not in *Stylosanthes hamata* cv Verano or *Stylosanthes scabra* cv Seca. **1** has not been detected in studies of water-stressed tropical grasses [3]. Further investigations on chickpea have shown that **1** occurs in both a free and combined form. Isolation of the latter is in progress.

EXPERIMENTAL

Plant material. Chickpea (*Cicer arietinum* (L.) cv Tyson) seeds were supplied and authenticated by Dr. D. F. Beech [4]. Plants were grown in large pots (100 × 25 cm i.d.) in a controlled environment (30°/25° day/night temp., 17 hr day). Pots were watered daily to a H_2O equivalent to pF_2. After 31 days, watering was ceased, and the last fully expanded leaves were harvested after 35 days without H_2O. H_2O potential of the harvested H_2O-stressed leaf material was −6.5 MPa. Leaf tissue was immediately frozen in dry ice, freeze-dried, ground (1 mm sieve) and stored at −20°.

General experimental conditions. MWs were measured by MS using field desorption techniques. GLC separations were effected on (a) 3% GE-SE 30 on Gas Chrom Q, stainless steel 1800 × 3 mm and (b) 12% HI-EFF 1BP on Gas Chrom P, nickel 1800 × 3 mm. Trimethylsilyl (TMSi) ethers were prepared using TMSi-imidazole [5]. Injector and detector temps were 220° and 285° respectively. Column temps were initially 140°, programmed to rise at (a) 2°/min to 190°, and (b) 4°/min to 190°. Carrier gas was N_2 at 25 ml/min. For TLC Eastman Chromagram sheets coated with Si gel (200 × 200 × 0.1 mm) were used, without activation, in solvent 1 ($CHCl_3$–MeOH, 17:3). Spots were visualized by spraying with (a) M $NH_2OH.HCl$ in MeOH–1.1 M KOH in MeOH (1:1), followed by (b) 2% $FeCl_3$ in 1% aq. HCl [1]. Reductions were carried out with aq. KBH_4 (0.5 ml, 1%) on 0.2 mg lactone at room temp. for 17 hr. Evapns were performed under red. pres. at less than 40°.

Isolation of the lactone. Leaf material (*ca* 2 g) was extracted with H_2O (3 × 50 ml) at 55° for 1.5 hr. The combined aq. solns were concd to a syrup, dried over P_2O_5 *in vacuo* and then treated with 3% MeOH–HCl (2 ml) at 55° for 4 hr. After removal of solvent, the residue was dissolved in 1 M KOH (2 ml) and allowed to stand for 17 hr. The alkaline soln was neutralized with 5 M HOAc and chromatographed on Bio Rad AG 1 × 8 (formate) ion exchange resin (100 × 10 mm). The resin was washed with H_2O (100 ml) followed by 2 M HCO_2H (100 ml). The lactone was recovered from the acid eluant. HCO_2H was evapd, and the lactone was chromatographed on Bio Rad AG 50 × 8 (H^+) cation exchange resin. The lactone eluted with H_2O and was further treated with trifluoroacetic acid (1 ml, 2 M, 120° for 1 hr). The acid was evapd, and the lactone rechromatographed on Bio Rad AG 1 × 8 (formate) resin. The lactone eluted with H_2O and was further purified on small columns of Merck Kieselgel 60 (twice, solvent 1), and finally isolated as a colourless syrup (10 mg), $[\alpha]_D$ −29° (*c* 0.8, MeOH), with no UV absorbance at 275 nm. GLC of the TMSi ether [column (a)] showed 2 peaks (*ca.* 4:1) at 4.0 and 6.2 min. After $NaBH_4$ reduction, the TMSi derivative gave a single peak at 8.1 min (cf. erythritol 6.8 min, xylitol 13.5 min). The underivatized lactone on column (b) gave a peak at 35.5 min; TLC R_f 0.34 ($CHCl_3$–MeOH, 17:3) [discrete pink spot with sprays (a) and (b)]. IR ν_{max}^{KBr} cm^{-1}: 3400, 2980, 2940, 1780, 1475, 1385, 1210, 1110, 1035, 980, 935, 875, 770, 745 and 720; ^1H NMR (100 MHz, $(CD_3)_2CO$): δ 4.44 (1H, *q*, $J_{3,2}$ = 10.5, 4.5 Hz, C-3), 4.11 (2H, *m*, $W_{1/2}$ = 12.2 Hz, C-4), 1.38 (3H, *s*, C-2 (Me)); ^{13}C NMR (25 MHz, $(CD_3)_2CO$): δ 178.1 (C-1), 74.1 (C-3), 73.8 (C-2), 72.2 (C-4), 21.6 (C–$\underline{C}H_3$); MS *m/z* (rel. int.): 132 (100), 131 (6), 104 (7), 103 (3), 87 (3). (Found: M^+, 132.04256. $C_5H_8O_4$ requires: M^+, 132.04225; *m/z*, 104.04693. $C_4H_8O_3$ requires: M^+, 104.04734).

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