A NEW LACTONE FROM WATER-STRESSED CHICKPEA

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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 diastereoisomic 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.

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 cajan), but not in Stylosanthes hamata cv Verao 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 x 25 cm i.d.) in a controlled environment (30°C/25°C day/night temp., 17 hr day). Pots were watered daily to a H₂O equivalent to pF 2. After 31 days, watering was ceased, and the last fully expanded leaves were harvested after 35 days without H₂O. H₂O potential of the harvested H₂O-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°C.
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 IBP 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°/rain to 190°, and (b) 4°/rain to 190°. Carrier gas was N₂ at 25ml/min. For TLC Eastman Chromagram sheets coated with Si gel (200 × 200 × 0.1 mm) were used, without activation, in solvent 1 (CHCl₃-MeOH, 17: 3). Spots were visualized by spraying with (a) M NH₂OH.HCl in MeOH-1% KH₂PO₄ in MeOH (1:1), followed by (b) 2% FeCl₃ in 1% aq. HCl [1]. Reductions were carried out with aq. KBH₄ (0.5 ml, 1%) on 0.2mg lactone at room temp. for 17hr. Evapns were performed under red. pres. at less than 40°.

Isolation of the lactone. Leaf material (ca 2 g) was extracted with H₂O (3 x 50ml) at 55° for 1.5hr. The combined aq. solns were cone'd to a syrup, dried over P₂O₅ in vacuo and then treated with 3% MeOH-HCl in MeOH-1 M KOH in MeOH (1:1), followed by (b) 2% FeCl₃ in 1% aq. HCl [1]. Reductions were carried out with aq. KBH₄ (0.5 ml, 1%) on 0.2mg lactone at room temp. for 17hr. Evans were performed under red. pres. at less than 40°.

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REFERENCES