

**Figures 1A & B.** A. Healthy pummelo fruit and its transverse section. B. Impietratura affected pummelo fruits and a transverse section showing albedo gumming.

of the disease were observed on a grape fruit tree at IARI orchard and recently in May, 1983 on four trees of Mosambi at Hyderabad.

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## CRYSTAL AND MOLECULAR STRUCTURE OF (*d*-LYSINATO) (*l*-LYSINATO) DICHLORO COPPER (II) DIHYDRATE

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THE crystal and molecular structure of dichloro (*d*-lysinate) (*l*-lysinate) copper (II) dihydrate has been

investigated as a part of our research programme on the structure and function of biomolecules. The compound was prepared by reacting DL-lysine hydrochloride with basic copper carbonate. Single crystals, suitable for x-ray work, were obtained by slow evaporation of the resulting blue solution over a period of days. Preliminary studies indicated the space group to be  $P2_1/n$  and density measurements showed that there are 2 molecules in the unit cell.

Diffraction data were obtained using an Enraf-Nonius CAD4 automated diffractometer with monochromatized Cu-K $\alpha$  radiation in the range  $4^\circ \leq 2\theta \leq 130^\circ$ . This yielded 1481 reflections of which 1191 had  $I \geq 3\sigma(I)$  and were considered observed. Accurate cell dimensions obtained by a least-squares fit of 25 arbitrarily chosen higher order reflections were:  $a = 5.152(1)$ ,  $b = 17.394(2)$ ,  $c = 11.325(2)$  Å and  $\beta = 96.81(1)^\circ$ .

The structure was solved by the heavy-atom method. A difference map, based on the positions of copper and chlorines, revealed all the non-hydrogen atoms; isotropic, followed by anisotropic refinement yielded an  $R$  value of 0.059. Inclusion of hydrogens and a mixed-mode refinement led to the final  $R$  value of 0.039 for all the observed data.

The coordination of the copper atom corresponds to a distorted octahedron with four short and two long bonds. The basal valencies are provided by the hydroxyl oxygens of the carboxyl group and nitrogens of the alpha-amino group (and their centrosymmetrically related partners) at distances of 1.937(1) Å and 1.970(2) Å respectively. These values can be compared with those reported for copper iminodiacetate dihydrate (2.014 and 1.945 Å, 1.995 and 1.961 Å, average  $\sigma = 0.008$  Å)<sup>1</sup>, bis (L-leucinato) copper (1.996 and 1.989 Å, 1.960 and 1.960 Å, average  $\sigma = 0.004$  Å)<sup>2</sup>, and *trans*-bis-(L-methionate) copper (2.01 and 1.97 Å, average  $\sigma = 0.02$  Å)<sup>3</sup>. The angle  $N_1CuO_1$  is  $84.6(1)^\circ$ . Chlorines approach the apical positions of the octahedron at distances of 3.047(1) Å. The angles  $N_1CuCl$  and  $O_1CuCl$  are  $90.5(1)^\circ$  and  $85.7(1)^\circ$  respectively. These observations show that the base is very nearly square-planar and the Cu-Cl vector is nearly perpendicular to  $O_1N_1CuO_1$  'N<sub>1</sub>' plane. The view of the molecule showing a minimum overlap is displayed in figure 1.

C(sp<sup>3</sup>)-C(sp<sup>3</sup>) distances in the lysine moiety range from 1.485(3) to 1.535(3) Å, while the C-N distances are 1.485(3) and 1.479(3) Å. The C-O distances are 1.230(3) and 1.270(3) Å, suggesting that this moiety is approaching a zwitterionic form. The distances and angles in the lysine moiety compare well with those

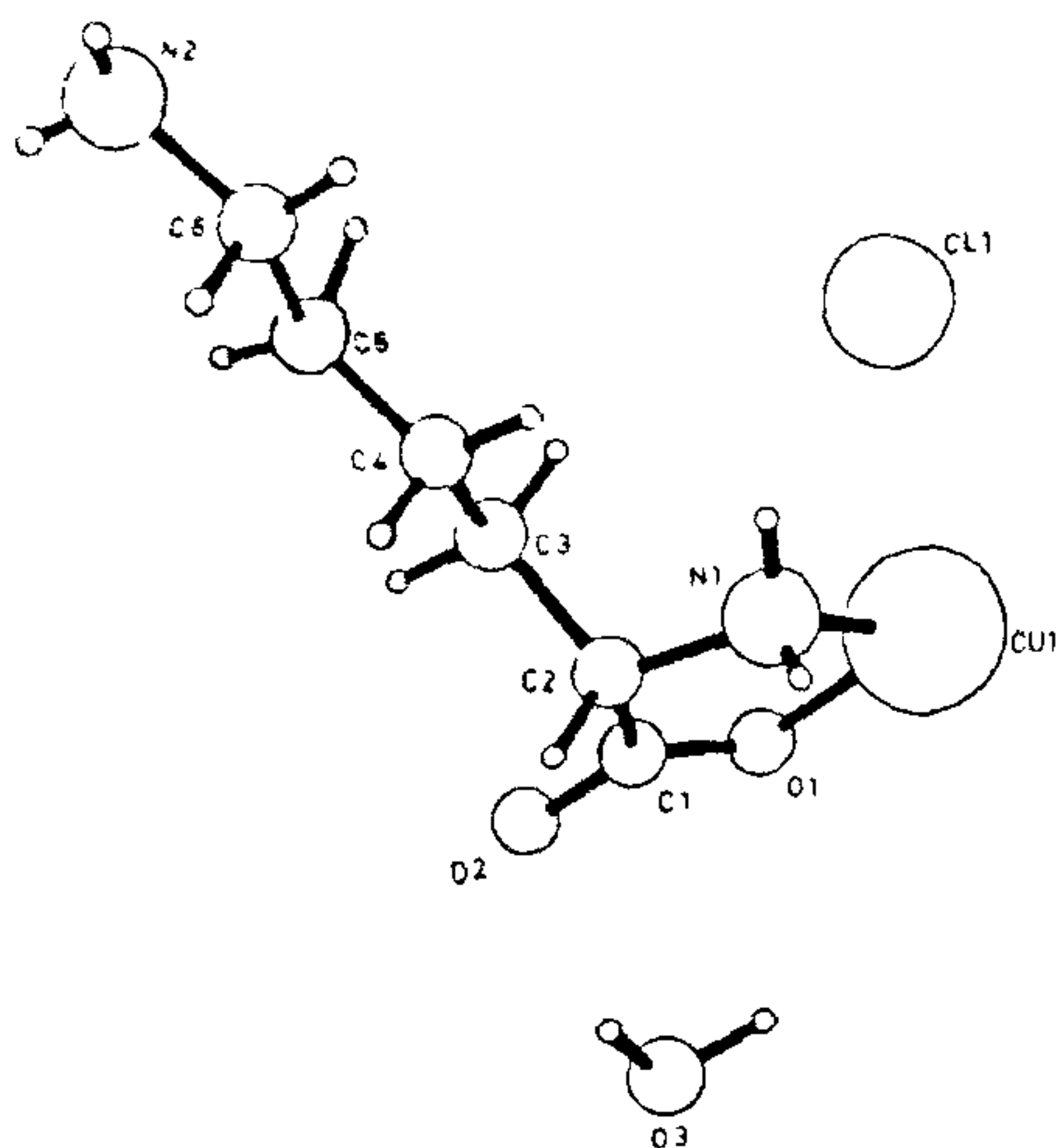


Figure 1. A minimum overlap view of the molecule (only one ligand and one water is shown)

reported for L-lysine hydrochloride<sup>4</sup>, DL-lysine hydrochloride<sup>5</sup>, L-lysine aspartate<sup>6</sup> and lysinato hexachloro platinum<sup>7</sup>. The C–O bond lengths in the platinum complex are, however, 1.310 and 1.235 Å, showing that the carboxyl group of the lysine moiety in this complex exists in the unionized form. Atom C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub> and N<sub>2</sub> are coplanar within 0.06 Å. The dihedral angle between the carboxyl group and the aliphatic chain is  $-65.2^\circ$ . The nitrogen N<sub>1</sub>, attached to the C(α) is displaced by 0.274 Å from the plane defined by C<sub>1</sub>C<sub>2</sub>O<sub>1</sub>O<sub>2</sub>. The lysine moiety in the copper complex exists in the extended form, with C<sub>5</sub> trans to C<sub>2</sub>, C<sub>6</sub> trans to C<sub>3</sub> and N<sub>2</sub> trans to C<sub>4</sub>, typical of a long chain aliphatic compound.

A view of the molecular packing along the *a* axis is shown in figure 2. The molecules are held in space by a network of hydrogen bonds. They are listed below:

(i) a N–H...Cl<sup>-</sup> bond of length 3.048 Å, connecting an alpha-nitrogen to a chlorine translated by a unit along *a* direction.

(ii) a N–H...Cl<sup>-</sup> bond of length 3.133 Å, connecting an ε-nitrogen to a chlorine related by a centre of symmetry.

(iii) a O<sub>3</sub>–H...Cl<sup>-</sup> bond of length 3.285 Å, connecting a water molecule to a chlorine related by a centre of symmetry.

(iv) a N–H...O<sub>1</sub> bond of length 3.023 Å, connect-

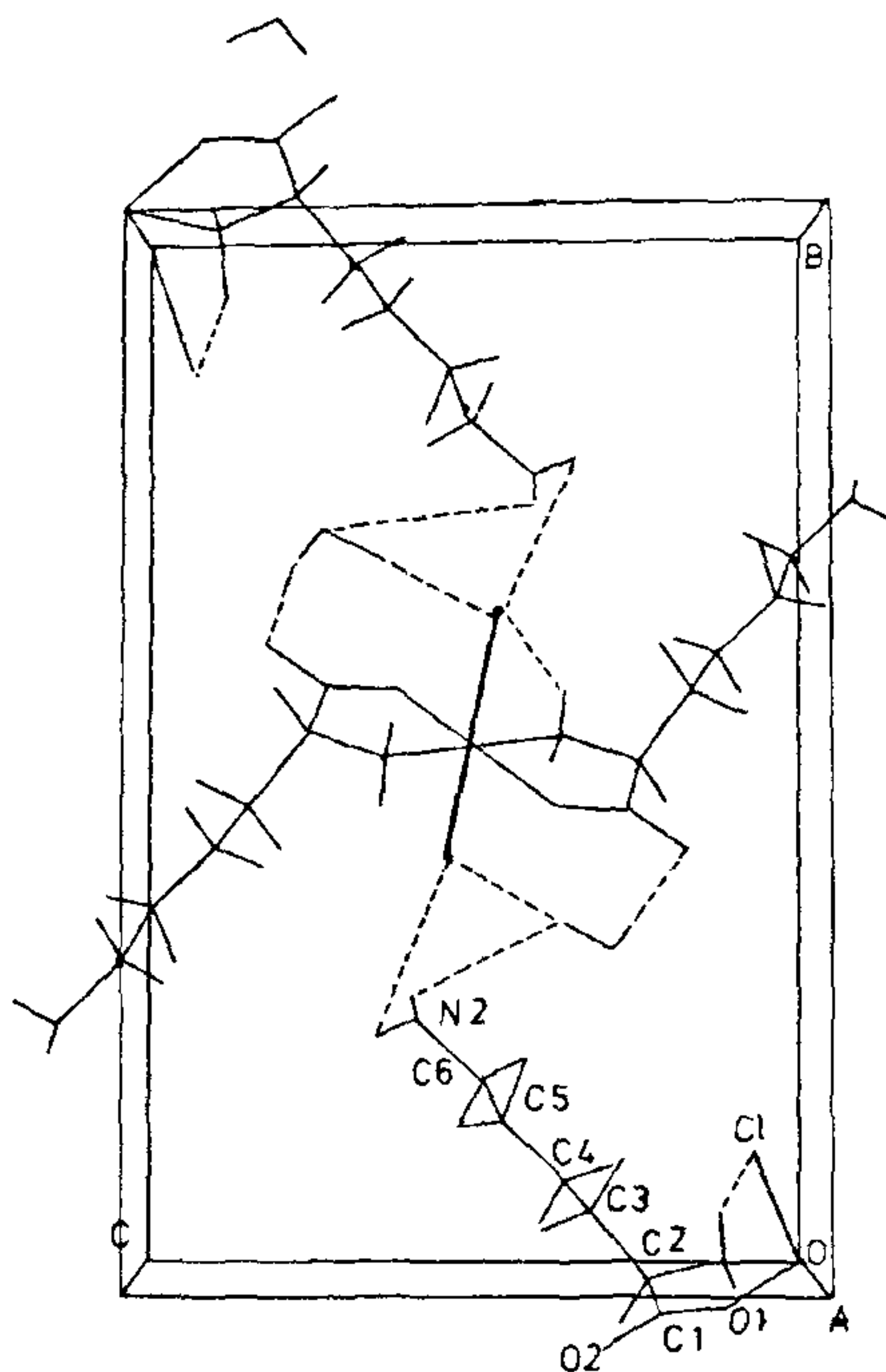


Figure 2. Structure viewed along the *a* axis.

ing an alpha-nitrogen to a hydroxyl oxygen displaced by a unit translation along *a*.

(v) a N–H...O<sub>3</sub> bond of length 2.802 Å, connecting an ε-nitrogen to a water O<sub>3</sub> related by a centre of inversion and

(vi) a O<sub>3</sub>–H...O<sub>2</sub> bond of length 2.928 Å, connecting a water oxygen to a carbonyl oxygen displaced by a unit translation along *a*.

Thus all the hydrogens available for bonding are utilized. The lengths of the hydrogen bonds indicate that these range from weak to moderate interactions.

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### SEED GERMINATION STUDIES WITH *CENCHRUS CILIARIS* L. II. ISOLATION AND CHARACTERISATION OF GERMINATION INHIBITORS FROM THE SPIKELETS

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IN *Cenchrus ciliaris* L, a prominent fodder grass of Indian arid zone, seeds (caryopsis) are enclosed within glumes in spikelets (dispersal unit) and the removal of caryopsis from the enclosing glumes has an enhanced effect on the per cent germination as compared to spikelets<sup>1-4</sup>. Inhibition of seed germination of the grass has been recorded by glume extract<sup>2,3</sup> and these inhibitors were believed to be coumarins<sup>2</sup>. Inhibition of seed germination by spikelet leachate was also recorded<sup>4</sup> and it seems that these inhibitors play an important role in restoring seed viability for a longer period. It was, therefore, decided to undertake the identification of the inhibitors associated with the dispersal unit of this range grass.

Spikelet leachate (obtained after soaking of spikelets in distilled water for 24 hr) examined for phenolics revealed the phenolic onium-ions (Anthocyanins<sup>5</sup>). Therefore, the spikelets were first extracted with petroleum ether to remove non-polar and non-phenolic substances and thereafter, the pigment was extracted in methanol-HCl (99:1 v/v) and purified chromatographically<sup>6</sup>. A part of the purified pigment was acid-hydrolysed and aglycone (anthocyanidin) was collected in amyl alcohol and purified. The aqueous layer of the hydrolysate contains HCl, besides

the sugar residue and before analysis the acid was removed from the sugar sample<sup>8</sup>. For determining the acyl residue of the pigment, spikelet leachate was hydrolysed with 2 NHCl for 5 hr at 100°C and filtered through Whatman No. 1 filter paper. The filtrate was then extracted<sup>9</sup> for phenolic substances soluble in ether and ethyl acetate.

The  $R_f$  values of the anthocyanin pigment, its aglycone and acyl residue were studied by descending chromatography on Whatman No. 1 paper. The solvents used for anthocyanin pigment were: BAW, *n*-butanol-acetic acid-water (4:1:5); Bu-HCl, *n*-butanol-2NHCl (1:1) and 1% HCl. The solvents used for the aglycone were: Forestal-acetic acid-conc. HCl-water (30:3:10); Formic-Formic acid-conc. HCl-water (5:2:3). The solvents used for acyl residue (caffeic acid) were: BAW, butanol-acetic acid-water (63:10:27) and IBW, isopropanol-butanol-water (140:20:60). The sugar fraction of the pigment was also identified chromatographically by studying the anilin oxalate complex<sup>10</sup> of the sugar<sup>11</sup>.

The results of the present work revealed that the  $R_f$  and  $\lambda_{max}$  values of the extracted pigment and its aglycone tallied closely with those of 3-monosidic glycoside of cyanidin and authentic cyanidin. The  $R_f$  ( $\times 100$ ) values for the pigment were 42 (BAW), 32 (Bu HCl) and 05 (1% HCl) and absorption maxima in Me-OH-HCl were 274 (UV) and 523 (visible) respectively. The values for the aglycone are 49 (Forestal) and 22 (Formic) and  $\lambda_{max}$  values 277 (UV) and 535 (visible) respectively. The  $R_f$  ( $\times 100$ ) value of the sugar is 33 (ethyl acetate-pyridine-water, 2:1:2) and the colour of its aniline oxalate complex<sup>11</sup> was similar to those of standard arabinose. Finally, the  $R_f$  ( $\times 100$ ) value of its acyl residue (caffeic acid) are 81 (BAW) and 72 (IBW), which gives brown colour with diazotised *p*-nitroaniline. The pigment fluoresces in UV light and gives dull magenta colour which may be considered as an additional proof for the pigment being a 3-glycoside-a monoside. Thus the inhibitor (pigment) has the following structural features. (1) The aglycone part of the pigment is cyanidin. (2) The pigment is a 3-glycoside-a monoside. (3) The sugar present in the pigment is arabinose. (4) Acylated residue is caffeic acid. Therefore, the pigment is cyanidin-3-arabinoside, acylated with caffeic acid.

Phenolics and coumarins are often reported as almost universally present inhibitors, which also act as germination inhibitors in seed husks, coats, fruits etc<sup>12</sup>. Flavonoids with a basic C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> skeleton are widespread in seed plants and both aglycones and glycosides are extremely potent toxins to seed germi-