conjugates added to pregnancy urine were gel-filtered on Sephadex G-25, and peaks I and II were collected separately and evaporated to dryness in vacuo. The dry residues were taken up in 3 ml of the eluting solvent and chromatographed in the systems designed for peak I and II conjugates. The presence of urinary peak I and II contents in the samples did not alter the elution pattern of the labeled

reference compounds.

Application. In chromatographic experiments on Sephadex LH-20 the elution pattern of labeled estriol glucuronides and estriol sulfate closely resembled that of the neutral steroid glucuronides and monosulfates. In addition, the estriol double conjugate (E₃-3S,16Gl) was strongly retarded in the column, as is the case with the neutral steroid disul-fates. 11,16 This seems to confirm earlier studies,8,9 which suggested that it is the mode of conjugation, rather than the steroid nucleus, which determines the elution behaviour of conjugated steroids on lipophilic Sephadex. Experiments with labeled estriol conjugates indicate that by chromatography on Sephadex LH-20 a convenient group separation of estrogen conjugates may be achieved, where at least glucuronides, sulfates, and sulfo-glucuronides can be separated from each other. In addition, gel filtration on Sephadex G-25 can be used in the separation of estrogen 3-glucuronides from estrogen 16-glucuronides. Thus it can be concluded that gel chromatography on Sephadex LH-20 in combination with gel filtration on Sephadex G-25 provides a useful means for group separation of urinary estrogen conjugates.

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Studies on Orchidaceae Alkaloids

XX.* The Constitution and Relative Configuration of Crepidine, an Alkaloid from *Dendrobium* crepidatum Lindl.

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Five closely related crystalline alkaloids have been isolated from *Dendrobium* crepidatum Lindl.² One of the bases (C₂₁H₂₉NO₃), named crepidine (I), crystal-

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^{*} No. XIX of this series, see Ref. 1.

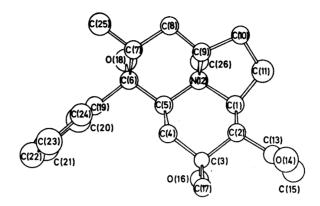


Fig. 1. A perspective view of crepidine methiodide (II).

lized from ethanol as colourless needles, m.p. $221-222^{\circ}$, $[\alpha]_D^{24}-78^{\circ}$ (c 0.50, chloroform). In this communication we report the constitution and relative configuration of crepidine (I), based on an X-ray diffraction investigation of the corresponding methodide (II).

The crystal structure of II ($C_{22}H_{32}INO_{3}$), m.p. $240-242^{\circ}$ (dec.), $\left[\alpha\right]_{D}^{24}-17^{\circ}$ (c 1.04, methanol), was determined by the X-ray single crystal technique using an automatic Siemens diffractometer. The compound crystallizes from acetone in the monoclinic space group $P2_{1}$ with one molecule per asymmetric unit. The cell dimensions are $a=15.307\pm5$ Å, $b=9.198\pm2$ Å, $c=7.842\pm5$ Å and $\beta=97.57^{\circ}+5$.

The x and z coordinates of the iodide ion were determined from a three-dimensional Patterson function. An arbitrary value of 1/4 was assigned to the y coordinate. The remaining non-hydrogen atoms were located from three-dimensional electron density maps. Successive Fourier calculations revealed the molecular structure, and the coordinates were refined by the full-matrix least-squares technique. The final reliability index was 0.044.

Fig. 1 shows a perspective view of the molecule. Bond lengths are given in Fig. 2. The deviations from normal values are consistent with the relatively large standard deviations calculated for the bond lengths in the molecule. The standard deviations are in the range 0.02-0.03 Å, which may be explained by the presence of the iodide ion with its predominant scattering power and by the fact that the crystals are unstable to X-rays. The

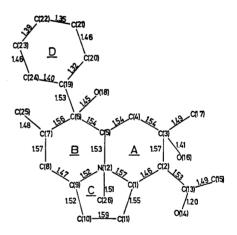


Fig. 2. Bond lengths in crepidine methiodide (II).

distance between the oxygen atom O(18) and the closest iodide ion is 3.54 Å, indicating a hydrogen bond $O-H\cdots I^-$.

Full details of the X-ray diffraction investigation will be published elsewhere.3

Treatment of crepidine methiodide (II) with sodium hydroxide (2 M, 25°, 30 min) afforded an amorphous base (III) exhibiting IR bands (CHCl₃) at 1720 cm⁻¹ (saturated ketone), 1675 and 1628 cm⁻¹ (α,β-unsaturated ketone). The molecular formula for III, C₂₂H₃₁NO₃, was determined from the integral of its NMR spectrum, and by mass spectrometry. The NMR spectrum of III (in pyridine-d₅) shows

signals at τ 9.10 (d, 3 H, J=6 Hz, CHC H_3), τ 8.33 (s, 3 H, C H_3 COCH $_2$ —), τ 7.82 (s, 3 H, NCH $_3$ or CH $_3$ CO—), τ 7.77 (s, 3 H, NCH $_3$ or CH $_3$ CO—), τ 7.55 (d, 2 H, NCH $_3$ or CH $_3$ CO—), τ 6.48 (t, 1 H, J=5 Hz, —COCH $_2$ CH—), τ 6.48 (t, 1 H, J=5 Hz, —COCH $_2$ CHN), τ 5.38 (s, 1 H, OH, concentration dependent) and τ 2.2—2.9 (m, 5 H, phenyl group). Two olefinic protons exhibits an ABX $_2$ pattern with τ_A 3.82 and τ_B 3.14 ($J_{AB}=16$ Hz, $J_{AX}=1.1$ Hz, $J_{BX}=6$ Hz) indicating the —CH $_2$ —CH $_B$ =CH $_A$ —CO— system. The large AB coupling implies a trans configuration for these protons.

HOW HOW CH₃

$$CH_3$$
 CH_3
 CH_3

Fig. 3. Alkaline degradation of crepidine methiodide (II).

A full report on the isolation and structural determination of the alkaloids found in *D. crepidatum* will be published later.

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Solute Alignment in the Nematic Mesophase

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The anisotropy in the solute-solvent intermolecular potential results in the partial alignment of non-spherical solutes by nematogenic solvents. The nature of the anisotropic potential is conveniently studied by measuring the ordering matrix \mathscr{O} which describes the solute alignment.^{1,2} The elements of the matrix are defined by

$$\sigma_{ij} = \frac{\overline{3l_il_j - \delta_{ij}}}{2}$$

where the bar denotes an ensemble average and l_* is the direction cosine between the *i*th molecular axis and the optic axis of the mesophase.³ When the solute is paramagnetic the ordering matrix may be determined from the electron resonance spectra of the solute, both above and below the nematic-isotropic transition point.⁴ The resulting shifts in the g factor and coupling constants are directly related to the elements of the ordering matrix by

 $\delta g = \sum_{i,j} g'_{ij} \mathscr{O}_{ij}$

and

$$\delta a = \sum\limits_{i,j} {A'}_{ij} \, \mathscr{O}_{ij}$$

where g' and A' are the anisotropic g and hyperfine tensors. In this communication we report such measurements for 2-substituted 4,4,5,5-tetramethyl-1,3-dioxy-imidazolines (I) which were prepared by employing the synthesis described by Osiecki and Ullman. 5

$$R = -(CH_2)_{AO} CH_3;$$

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