protein. The spectral features, characterized by large positive ellipticities, are by no means reminiscent of those of CB-Lac or even reduced-reoxydized α-lactalbumin. Most probably the coupling of the cysteine side chains is not the correct one but proceeds randomly yielding different molecular species. When the reoxidation of the mixture was carried out at lower concentration of the catalyst (5×10^{-5} *M* $CuSO_4$), quite different CD spectra were obtained (Figure 3). Although the spectra at various stages of the reoxidation process are still distinct from those of CB-Lac and even more from those of reduced-reoxidized α-lactalbumin, nevertheless they show a tendency towards features clearly reminiscent of the protein containing the correct disulphide bonds. Apparently, judging from the CD standpoint, some of CB-1 and CB-2 molecules interact together by restoring, through a correct coupling of sulphydryl groups, at least part of the non-covalent interactions determining the original structure. It is to be noted that an equimolar mixture of carboxymethylated CB-1 and CB-2 (in which disulphide formation is prevented) give a CD spectrum comparable to the sum of the curves of the individual peptides. The role played by the catalyst, Cu^{++} ions, deserves further comment. It is reasonably anticipated that the correct refolding of the polypeptide chain is initiated by the regeneration of a part of original disulphide bonds. Of course various kinds of mismatched disulphide bonds are possible: however, they could be corrected, at least partially, via disulphide interchange. In the presence of excess copper ion, the rate of reoxidation may be too high to allow preferential formation of the disulphide bond(s) which initiates the correct refolding. In this case disulphide bonds may be formed incorrectly, thus producing stable molecular species completely different from the native protein. A similar influence of the catalyst concentration has been observed in the reoxidation of reduced Taka-amylase A.[10].

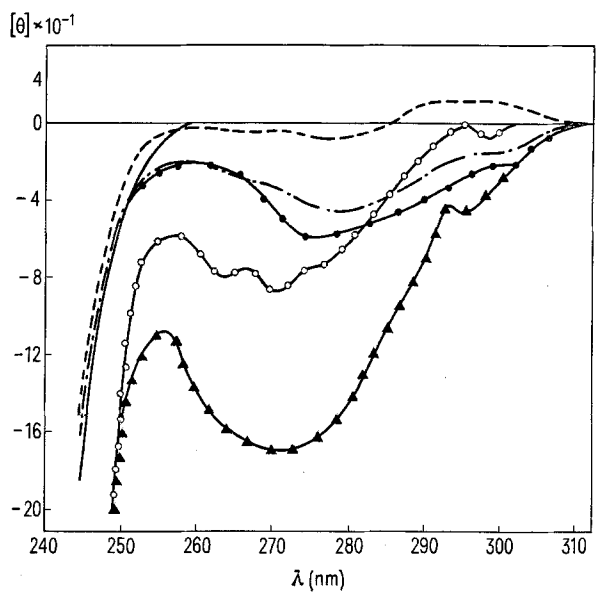

Fig. 3. Near-UV-CD-spectra of the equimolar mixture CB-1 + CB-2 in 0.025*M* *Tris*-Cl buffer, pH 8.0, containing 5×10^{-5}*M* $CuSO_4$: (---), after 1 h 30 min of reoxidation; (-·-·-), after 18 h 20 min; (-●-●-), after 168 h 20 min. (-▲-▲-), reduced-reoxidized α-lactalbumin. Other symbols as in Figure 2.

Riassunto. Si é studiato, mediante dicroismo circolare, il processo di riossidazione-ricombinazione di due frammenti ottenuti per attacco con Br-CN e successiva riduzione dell-α-lattalbumina. Si é trovato che la concentrazione del catalizzatore $CuSO_4$ svolge un ruolo importante nel dirigere il riaccoppiamento dei gruppi SH.

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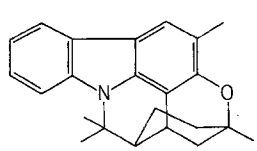
[11] To whom reprint requests should be addressed.

The Crystal Structure of Murrayazoline (Mahanimbidine, Curryangin)

In connection with an investigation on carbazoles of the Rutaceae, there was isolated a new alkaloid, murrayazoline, $C_{23}H_{25}NO$ (M^+ 331), mp 260–262°, from an alcoholic extract of the stem-bark of *Murraya koenigii* Spreng[1]. The UV-spectrum was suggestive of the 2-methoxy carbazole chromophore [λ_{max} 245 nm and 307 nm (log ε 4.69 and 4.16)][2,3]. Further studies revealed that like the known mahanimbine[4], murrayazoline was derived from a 2-hydroxy-3-methyl carbazole to which a monoterpene (C_{10}-unit) fragment was fused at the 2-position through an ether linkage.



I

This alkaloid was also obtained from the leaves and the stem bark of the plant by two other groups independently, and renamed mahanimbidine[5] and curryangin[6,7]. On the basis of additional chemical and spectral data, the structure I (9a, 10, 11, 12, 13, 13, 13a-hexahydro-2, 9, 9, 12-tetramethyl-1, 12-epoxy-9*H*-indolo [3, 2, 1-*de*]phenanthridine) was assigned to the compound. It was felt that an independent proof of the hexacyclic system would be of value, especially in view of the biological activity reported for various carbazoles[8–10].

Suitable, colorless crystals of I were obtained by a slow evaporation of an acetone solution. One Å intensity data (maximum sin $\theta/\lambda = 0.5$) was collected with copper radiation on a Syntex P1 diffractometer equipped with a graphite monochromator. The intensity data was processed and all subsequent crystallographic calculations were performed using subprograms of the CRYM system[11]. Phasing was accomplished by a routine application of symbolic addition[12]. A trial structure was obtained from

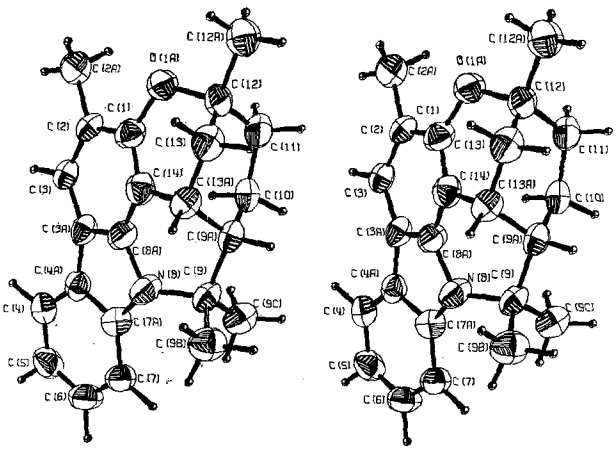
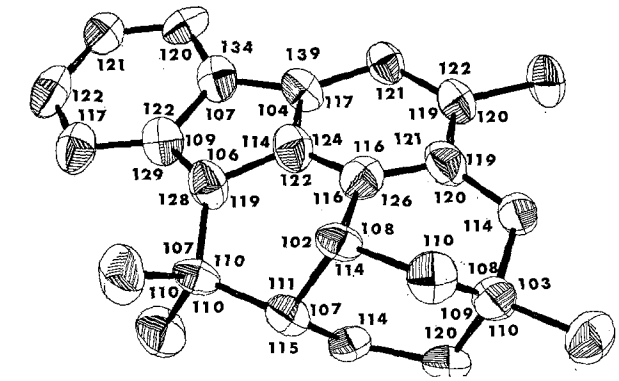


Fig. 1. Stereoplot of the molecule.

Molecular formula	$C_{23}H_{25}NO$(331.46)
Crystal size	$0.10 \times 0.15 \times 0.20$ mm
Cell dimensions	a = 11.19 (1) Å
	b = 11.05 (1) Å
	c = 15.65 (2) Å
	β = 115.27 (5) Å
Space group	$P2_1/c$ (4 molecules/unit cell)
Density obsd.	1.24 g/cm^3
Density calcd.	1.258 g/cm^3
Scan mode	$\theta/2\theta$
Scan rate	2°/min in 2θ
Background count time	1.0 (scan time)
Total reflections	1803
Non-zero reflections	1500



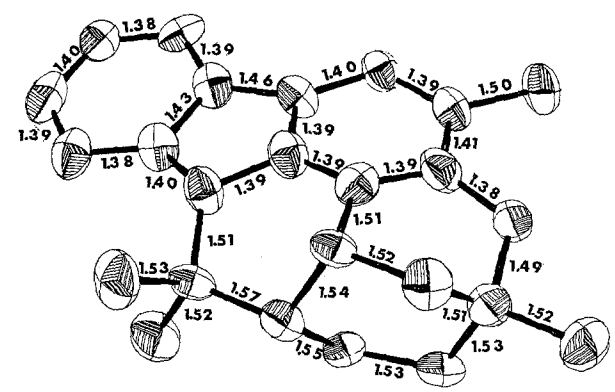


Fig. 2. Bond distances (Å) and angles (degrees). The uncertainties in the C, N, O distances are about 0.01 Å. Uncertainties in the bond angles are about 0.5°. Angles not shown on the drawing are 9B-9-9A, 111°; 8-9-9C, 110°; 13-12-12A, 114°; 1A-12-11, 112°; 4-4A-7A, 119°.

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the first E map and refined to an R index of 0.074. Hydrogen positions were located using difference Fourier techniques, but these positions were not subjected to refinement. The final cycles of full matrix least-squares refinement contained the coordinates of the non-hydrogen atoms in one matrix and the scale factor and anisotropic temperature factors in a second matrix. The calculated shifts in the final cycle of refinement were all less than $^1/_{50}$ of the standard deviation. A final difference Fourier revealed no missing or misplaced atoms. Crystal and data collection parameters are summarized in the Table.

The refined coordinates were stereographically plotted[13] and are shown in Figure 1. The bond distances and angles are summarized in Figure 2. Full details of the analysis may be obtained from the first author[14].

Riassunto. La struttura del murrayazoline, un' alcoloide carbazole, é stata confermata per via cristallografica con i raggi X.

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