trols gave 2.0% material in the "sterol" region with the remainder recovered as unchanged oxides.

The glycols formed enzymically from IIIa and IIIb gave pure acetonides indistinguishable on tlc and glpc from the acetonides VIa and VIb, respectively.

The materials obtained from IIIa and IIIb that moved in the sterol region on tlc were analyzed by glpc on SE-30 with collection of fractions corresponding to nonenzymic (R_c 2.1) and enzymic (sterol) products (R_c 2.1–4.1; R_c for lanosterol, 3.04). Only the *trans*-oxide IIIb yielded a product, IV, in the latter region (R_c 2.54). Essentially all recovered radioactivity in the case of IIIa, as in the boiled enzyme controls, was in the nonsteroidal region (R_c 2.1).

The structure assignment of IV rests on the following observations. The biosynthetic material (as the methyl ether) has R_c 4.52 on DEGS, in close agreement with a calculated value based on retention times of a structurally related series of compounds.[14] On hydrogenation followed by equilibration with dry HCl in $CHCl_3$ IV yielded two products identical in retention times and in molar proportions with those similarly obtained from authentic 4α,14α-dimethyl-Δ⁷-cholesten-3β-ol (VIII)[15] (*i.e.*, 3:1 Δ⁷–Δ⁸, separated on DEGS as the methyl ethers, with R_c 4.07 and 3.12, respectively). Labeled material corresponding to the Δ⁷ derivative VIII was collected from glpc on SE-30 with R_c 2.45–2.95 and cocrystallized with a synthetic sample of VIII as the free sterols and the acetates with no loss of specific activity (sterol, 289–297 and acetate, 238–246 dpm/mg).

The 4α-methyl assignment to IV, rather than 4β-, is to be expected if the stereochemical fate of the methyl group of IIIb is the same as that of the corresponding methyl group in squalene 2,3-oxide. It is supported by the identical behavior on glpc on SE-30 of authentic VIII and the hydrogenated and isomerized product from IV, since we have confirmed[17] the separation reported[18] for 4α- and 4β-methyl sterols on SE-30 (though not on DEGS).

We have considered the possible formation of an alternative product such as the A-nor steroid XI, which should be intermediate in polarity between the sterols and glycols (R_f 0.17–0.37 on tlc). In the ketone derived from XI the labeled hydrogens are β to the keto group and thus, in contrast to those in the ketone corresponding to IV, should be retained under enolizing conditions. Oxidation of material of R_f 0.17–0.37 followed by acid-catalyzed exchange and partition of the products between water and hexane rendered 97–98% of the label water soluble. Moreover no evidence of the formation of products of type XI was revealed by glpc analysis.

These results demonstrate the enzymic cyclization of the *trans*-oxide IIIb, in 1–1.5% yield, to 4α,14α-dimethyl-Δ⁸,²⁴-cholestadienol (IV). The only other identified enzyme product from IIIb was the glycol Vb. The hypothetical alternative cyclization product, the A-nor steroid XI, if formed, can only be present in much smaller amounts. The finding that, in contrast to the *trans*-oxide, the *cis*-oxide IIIa was not measurably cyclized prompts a variety of speculations concerning hitherto unrecognized steric and electronic determinants of the cyclization process, but these can only be fully elucidated by further experiments. Enzymic conversion of IIIb to IV is only 6% of the conversion of squalene 2,3-oxide to lanosterol under identical conditions. Thus, the *gem*-dimethyl structure of the natural substrate is of prime importance in the initiation of enzymic cyclization. The markedly different yields of glycols from IIIa and IIIb, though possibly of significance in relation to cyclase activity, require further study, since preliminary results suggest the presence of a separate oxide hydrolase system.

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(14) R. B. Clayton, *Biochemistry*, 1, 357 (1962).
(15) Synthesized from 14α,Δ⁷-cholesten-3β-ol (IX) (kindly supplied by Dr. John Knight) by the methods used by Neiderhiser and Wells for the preparation of 4α-methyl-Δ⁷-cholestenol (X).[16]
(16) D. H. Neiderhiser and W. W. Wells, *Arch. Biochem. Biophys.*, 81, 300 (1959).
(17) We thank Dr. J. L. Gaylor for a sample of authentic 4β-methyl-Δ⁷-cholestenol and Mr. K. B. Sharpless for samples of 4α- and 4β-methylcholestanols used in these studies.
(18) W. L. Miller, M. E. Kalafer, J. L. Gaylor, and C. V. Delwiche, *Biochemistry*, 6, 2673 (1967).

A Double Octant Rule for Planar Transition Metal Ion Complexes

In Table I are shown the results of circular dichroism measurements for divalent cupric and nickel ion complexes of tripeptides composed of glycyl and L-alanyl residues. The solutions measured were sufficiently basic that both amide hydrogens had ionized in all tripeptide complexes[1] with the result that amide nitrogens rather than amide oxygens are bound to the metal ions. Numerical values in Table I are the differences in molar absorptivities between left and right circularly polarized light at about 560 mμ for the cupric ion complexes[2] and at about 480 mμ for the yellow square-planar divalent nickel ion complexes. A set of measurements similar to those reported in Table I with leucine substituted for alanine shows near additivity with cupric ion but not with nickel ion.

Table I. Differential Molar Absorptivities of Divalent Metal Ion Complexes of Tripeptides Composed of L-Amino Acid Residues

Tripeptide	Cu[a]	Ni
Gly-Gly-Ala	−0.48	−0.85
Gly-Ala-Gly	−0.75	−1.12
Ala-Gly-Gly	−0.19	−0.11
Sum	−1.42	−2.08
Ala-Ala-Ala	−1.03	−2.10

[a] From ref 2.

(1) R. B. Martin, M. Chamberlin, and J. T. Edsall, *J. Am. Chem. Soc.*, 82, 495 (1960).
(2) G. F. Bryce and F. R. N. Gurd, *J. Biol. Chem.*, 241, 1439 (1966).

Simple octant rules have been proposed to account for the results of Cotton effect signs in the visible absorption region of transition metal ion complexes.[3,4] Simple octant rules cannot, however, account for the constant signs of the visible circular dichroism spectra of the square-planar tripeptide complexes reported in Table I. Wherever the nodal surfaces are drawn, simple octant rules predict that for each metal ion the Gly-Ala-Gly complexes in Table I should exhibit signs opposite those of the other two tripeptide complexes.

In order to account for the constant sign and near additivity of the differential molar absorptivities in Table I, a double octant rule is proposed where the plane of the metal ion is divided perpendicularly into eight sectors of alternating sign and approximately equal size. Below the plane the signs are opposite to those above in the same sector yielding 16 regions in all. Figure 1 shows division of a square-planar metal complex into eight sectors. The signs have significance

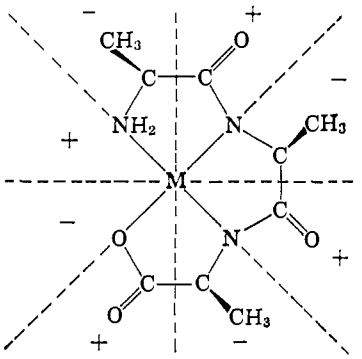

Figure 1. Signs of upper half of double octant above plane of chelate ring with structure of chelated tripeptide composed of L-alanyl residues.

only for the part of the sector above the plane; below the plane the signs are reversed. For the L-alanine-containing tripeptide complexes of Table I, the methyl groups of the side chain will be above the plane and the signs are chosen so as to indicate directly the signs of the differential molar absorptivities presented in Table I.

The double octant rule for planar transition metal ion complexes proposed here is identical with that indicated for molecules with D_{4h} symmetry.[5] Though the environment about the metal ion in Figure 1 only approximates this symmetry, the similarity of nitrogen and oxygen donors (tripeptide amides yield similar results) and the relative rigidity of the tripeptide complexes evidently make the pseudo-scalar representation for the D_{4h} point group a useful model. In the D_{4h} point group, two of the three d–d transitions are magnetic dipole allowed for each metal ion.

Without assuming any special puckering of chelate rings, the proposed double octant rule implies less about origins of circular dichroism in complexes containing optically active ligands than do the formulations of simple octant rules. Puckering may be invoked, however, to account for the lesser magnitudes in Table I for the N-terminal alanyl peptides where the greater flexibility of a tetrahedral nitrogen compared to trigonal nitrogens permits the side chain to take a position nearer the plane of the chelate ring.

A simple octant rule divides the plane of the complex into four quarters of alternating sign and would be unable to account for constant signs in Table I. The proposed double octant rule accounts for the identical signs as Figure 1 demonstrates. Bidentate chelates would in general obey either rule because they do contain substituents in octants of opposite sign as may be visualized by removing the central amino acid residue from Figure 1 and preparing octants by eliminating either pair of two alternate dotted lines and assigning opposite signs to adjacent octants. It is the additional substituent afforded by chelates such as tripeptides which provides a critical test for the superiority of the double octant rule for planar or near-planar complexes.

In applying octant or double octant rules, it is important to compare the circular dichroism (CD) signs for the same d–d transitions when comparing differing complexes. A 2:1 complex of a typical amino acid such as leucine with cupric ion exhibits, similar to those reported for other amino acids,[6] a small positive CD at >700 mμ, and a negative CD at about 610 mμ, corresponding to at least two transitions. A 2:1 histidine–cupric ion complex also exhibits two CD peaks of opposed sign, a positive one at 695 mμ and a small negative one at 560 mμ. Since the bis-histidine complex contains four nitrogen donors while the bis-leucine complex contains only two nitrogen donors, a shift to shorter wavelengths is expected in the histidine case, and the signs for the same d–d transitions appear identical for the two amino acid complexes. In addition positive and negative CD peaks of the 1:1 complex of histidine and cupric ion are only slightly shifted to shorter wavelengths compared to the 2:1 complex of leucine and cupric ion with the same number of nitrogen donors. Thus, provided no interchange of energy levels has occurred, when the same transitions are considered, tridentate chelates such as histidine appear to yield the same CD signs as bidentate amino acid chelates. Because of the difficulty of identifying transitions when more than one is contained within an absorption band, no simple diagnostic test for apical chelation as has been proposed[4] seems possible, especially when the number of coordinated nitrogen donors is also changing. Some apical chelation is expected in the 2:1 aspartic acid–cupric ion complex of two nitrogen donors. The CD spectra is similar to but weaker than that of the leucine complex except for the addition of a small positive peak at 560 mμ, which may be indicative of a tridentate ligand.

The only 2:1 L-amino acid–cupric ion complexes which we have discovered that possess positive signs for CD of the 2N, 2O donor transition near 610 mμ in leucine are proline and hydroxyproline. This result can be accommodated by noting that the C_5 carbon of the L-proline ring falls into a positive region of Figure 1. This perturbation is canceled by a below-the-plane

(3) C. J. Hawkins and E. Larsen, *Acta Chem. Scand.*, **19**, 185 (1965).
(4) K. M. Wellman, W. Mungall, T. G. Mecca, and C. R. Hare, *J. Am. Chem. Soc.*, **89**, 3647 (1967); *Chem. Eng. News*, 48 (Oct 2, 1967).
(5) J. A. Schellman, *J. Chem. Phys.*, **44**, 55 (1966).
(6) T. Yasui, *Bull. Chem. Soc. Japan*, **38**, 1736 (1965); T. Yasui, J. Hidaka, and Y. Shimura, *J. Am. Chem. Soc.*, 87, 2762 (1965).

methyl group in N-methyl-L-proline with the result that the CD spectrum of this 2:1 cupric complex is virtually identical with that of the L-leucine complex. It is difficult to see how these results can be accounted for by any chelate ring puckering hypothesis and a simple octant rule.[7]

(7) The ideas expressed in this paper have been employed over the past year and a half to systematize the results obtained by Dr. John M. Tsangaris and Dr. Joyce Wen Chang, both of whom submitted their Ph.D. theses in Aug 1967. Descriptions of their work have been submitted for publication.

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N-Carboxylic Acid Esters of 1,2- and 1,4-Dihydroquinolines. A New Class of Irreversible Inactivators of the Catecholamine α Receptors and Potent Central Nervous System Depressants

Sir:

We wish to report the discovery of the potent central nervous system depressant activity of certain N-carboxylic acid esters of 1,2- and 1,4-dihydroquinolines (I, II, and IV), a structural pattern without tangible analogy among drugs acting on the central nervous system (CNS). In addition, we have observed that several members of this new class of drugs are potent and selective irreversible inhibitors of the adrenergic α receptor, a feature that adds a new dimension to current theories of the molecular mechanisms of drug action at that level.[1-3] Until now, irreversible inhibition of the adrenergic α receptor could be achieved only with alkylating agents of the nitrogen mustard type of drugs (*i.e.*, dibenamine and its relatives[4,5]) which, however, do not possess CNS depressant activity.

These compounds were prepared from the appropriate quaternized quinolines as shown in Scheme I.[6] The 1,4-dihydro analog IV was also prepared by an alternate procedure. The starting material was *o*-nitrocinnamaldehyde diethyl acetal[7] which was hydrogenated (Raney nickel) to the corresponding saturated amine followed by acylation and ring closure to compound III under mild conditions of solvolysis.[8]

When compounds I and IV were administered in low oral doses to animals, they produced a progressive decrease in responsiveness and in locomotor activity, while at higher doses a state of cataleptic immobility ensued. This pharmacological profile is typical of all the drugs classified as major tranquilizers.[9] The pseudo-base II is pharmacologically equivalent to I and IV except for its low oral potency due to instability in the gastrointestinal tract. In the presence of dilute mineral acid it is rapidly decomposed to quinoline, carbon dioxide, and alcohol.

In anesthetized animals, compounds I, II, and IV induced a protracted blockade of the adrenergic α receptors that could not be reversed by massive doses of epinephrine (see Table I). A similar effect was also observed in isolated tissues where compound II was approximately 50 times more potent than either I or IV.

Scheme I

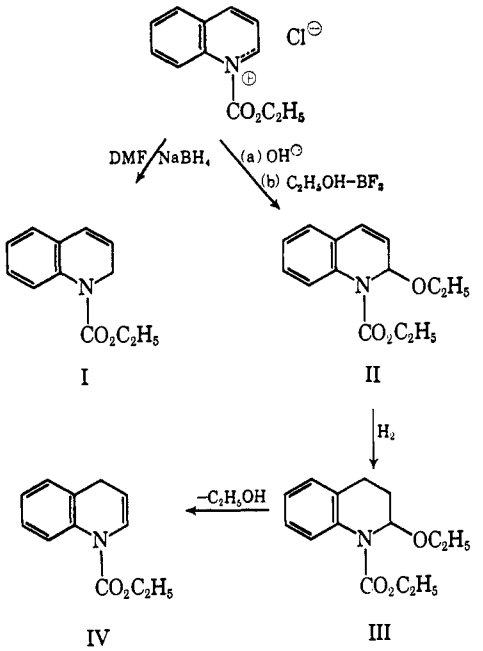

Table I. Relative Potencies of Compounds I, II, IV, and Chlorpromazine (CPZ)

Test	Potency (compound I = 1) I	II	IV	CPZ
CNS depressant[a]	1.0	1.5	0.2	0.4
Blockade of epinephrine 1 mg/kg iv[b]	1.0	6.0	1.0	Reversible

[a] Measured in rats by the cataleptogenic test of H. Fujimori and D. P. Cobb, *J. Pharmacol. Exptl. Therap.*, **148**, 151 (1965). [b] Determined on the blood pressure of anesthetized cats (pentobarbital).

From a large number of derivatives that were tested, the following significant trends in the structure–activity relationship emerged. Maximum activity was associated with short alkyl chains in the carbamate moiety. Substituents other than alkoxy or alkylthio at position 2 abolished activity, while substitution at positions 3, 4, and 8 was markedly detrimental to activity. The tetrahydro analogs as well as the dihydroisoquinoline isomers were devoid of activity.

The irreversible nature of the blockade obtained with I, II, and IV suggests that a covalent linkage may be

(1) B. Belleau, *Pharmacol. Rev.*, **18**, 131 (1966).
(2) B. M. Bloom and I. M. Goldman, *Advan. Drug Res.*, **3**, 121 (1966).
(3) B. Belleau, *Ann., N. Y. Acad. Sci.*, **139**, 580 (1967).
(4) M. Nickerson, *Pharmacol. Rev.*, **11**, 443 (1959).
(5) B. Belleau, *Can. J. Biochem.*, **36**, 731 (1958).
(6) All the compounds had correct empirical compositions and were further characterized by uv, ir, and nmr spectroscopy.
(7) J. Klein and E. D. Bergmann, *J. Am. Chem. Soc.*, **79**, 3452 (1957).
(8) The only 1,4-dihydroquinoline amide derivative (N-benzoyl) reported in the literature has been claimed by C. M. Knowles and G. M. Watt, *J. Am. Chem. Soc.*, **65**, 410 (1943). However, evidence will be presented in our full paper that their structure assignment is erroneous.
(9) P A. J. Janssen, *et al.*, *Arzneimittel-Forsch.*, **15**, 104 (1965).