Groups of 5 mice were injected ip with increasing doses of 1 and 2 in DMSO (0.1 ml/30-g mouse). After 10 days the LD_{50} was calculated using a maximum likelihood probit analysis method programmed for digital computation.

Groups of 16 mice were injected ip with increasing doses of 1 and 2 in propylene glycol (0.15 ml/30-g mouse). The mice were placed in individual, perforated, plastic centrifuge tubes, positioned on a rotating turntable, and (15 min after injection) exposed to 750 R of whole-body X irradiation (250 DVP, 15 mA, target-skin distance 50 cm, dose rate 45 R/min). Controls were exposed simultaneously with test doses of drugs. Mortality from day 3 to day 30 was tabulated. Propylene glycol was used as drug solvent for radiation studies instead of DMSO since the latter solvent has radioprotective properties of its own.

Relative metHb levels were determined with the Storer and Coon[1] modification of the method of Evelyn and Malloy[9] on a Spectronic 20 colorimeter.

Absolute Hb was determined on a Beckman DU using the Hycel reagent.

Animals were exposed to hyperbaric O_2 in a Bethelehem Corporation Hyperbaric Chamber Model 615 HP.

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Absolute Configuration of (+)- and (−)-*trans*-2-Phenylcyclopropylamine Hydrochloride†

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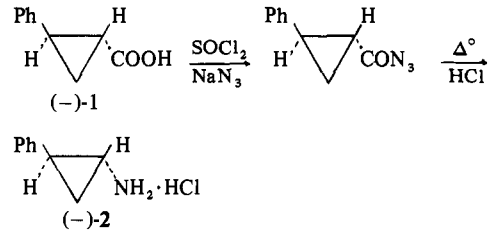
Received May 31, 1972

Tranylcypromine·HCl (**2**, *trans*-2-phenylcyclopropylamine·HCl) is a clinically useful agent in the treatment of mental depression and certain phobic-anxiety states. The therapeutic actions of this drug have been ascribed to its ability to inhibit monoamine oxidase. The inhibition of MAO by 2-phenylcyclopropylamine·HCl is characterized by a significant but low order of stereoselectivity with the trans isomer being three times more potent than the cis isomer and (+)-**2** being four times more potent than (−)-**2**.[1]

In view of the utility of the knowledge of absolute configuration in elucidating the nature of drug-biomolecule interactions,[2] we have undertaken the determination of absolute configuration of the tranylcypromine enantiomers.

(−)-Tranylcypromine·HCl was unequivocally shown to possess 1*R*:2*S* stereochemistry by virtue of its synthesis from (−)-1*R*:2*R*-2-phenylcyclopropanecarboxylic acid[3]



(**1**). The synthetic sequence employed is that of Burger and Yost.[4] It is of interest to note that the Curtius rearrangement of the acid azide to the isocyanate involves a transformation affecting the C-1 asymmetric center. However, the retention of the configuration of the migrating group in this rearrangement is well documented.[5]

Experimental Section

Melting points were taken on a Mel-Temp and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 114 polarimeter and a 1-dm cell. Ir (Perkin-Elmer 257) and nmr (Jeolco C-60HL) for all compounds were as expected.

Resolution of *trans*-2-Phenylcyclopropanecarboxylic Acid. A literature procedure[6] for the resolution of racemic 1 involves the use of brucine as a resolving agent. Experiments in our laboratory indicated that (+)-dehydroabietylamine was a superior resolving agent with regard to obtaining (−)-1 of high optical purity. The following procedure is illustrative of a typical resolution.

A warm soln of 8 g (0.049 mole) of (±)-1 in 50 ml of MeOH was slowly added to 13.9 g (0.049 mole) of (+)-dehydroabietylamine in 40 ml of warm MeOH. The mixt was allowed to sit at room temp, and the crystals were filtered, mp 159–167°. After three recrystn from aqueous MeOH (10–15%), 5.8 g of (−)-dehydroabietylamine 2-phenylcyclopropanecarboxylate was obtd, mp 174–174.5°; $[\alpha]^{25}D$ −80.2° (*c* 1, MeOH). The free acid was liberated from the salt by treatment with a satd soln of Na_2CO_3, extn with Et_2O, acidification of the aqueous fraction with cold, concd HCl, and filtration of the ppt to yield 1.3 g of (−)-1 after one recrystn from Me_2CO, mp 47–49°; $[\alpha]^{25}D$ −381.1° (*c* 1, $CHCl_3$) (lit.[6] mp 51–52°; $[\alpha]^{24}D$ −368° (*c* 0.931, $CHCl_3$)).

(−)-1*R*:2*S*-2-Phenylcyclopropylamine·HCl.[4] Thionyl chloride (5.96 g, 0.050 mole) was added dropwise to (−)-1 (4 g, 0.025 mole) at 0°. The soln was stirred at room temp for 24 hr, and the excess $SOCl_2$ was evapd *in vacuo*. The residue was dissolved in 60 ml of dry Me_2CO, and 10 ml of an aqueous soln of NaN_3 (2.88 g, 0.044 mole) was added dropwise with stirring at 0°. The mixt was stirred for 30 min and extd with toluene. The toluene soln was dried (Na_2SO_4) and dropped into a flask heated on a steam bath. After N_2 evolution had ceased the toluene was evapd *in vacuo*. The residue (isocyanate) was dissolved in 35% HCl, refluxed for 13 hr, concd *in vacuo*, and basified with 50 ml of 8 *N* NaOH. The mixt was extd three times with Et_2O, the ethereal fraction dried (Na_2SO_4) and concd *in vacuo*, and the residue distilled to afford 2.4 g of the amine, bp 68–70° (0.9 mm), $[\alpha]^{25}D$ −115.8° (*c* 1.13, $CHCl_3$). Treatment of the amine with ethereal HCl provided (−)-**2**, mp 176–178°; $[\alpha]^{25}D$ −67.7° (*c* 0.882, H_2O) (lit.[1] mp 180–181°; $[\alpha]^{25}D$ −75.5° (*c* 1, H_2O)).

Results and Discussion

The synthesis of (−)-2-phenylcyclopropylamine·HCl from (1*R*:2*R*)-2-phenylcyclopropanecarboxylic acid, as described, unequivocally establishes the absolute configuration of the enantiomers of tranylcypromine·HCl. Hence the more active MAO-inhibitory enantiomer of this agent possesses the 1*S*:2*R* configuration.

It is of interest to note that the weak MAO inhibitor, amphetamine (**3a**), and the potent inhibitor, 1-phenyl-2-propylhydrazine (**3b**, pheniprazine), possess the basic β-phenethylamine moiety and a chiral center α to the amino group as found in tranylcypromine. Further, as in the case of tranylcypromine, studies have shown that the greater MAO-inhibitory activity of amphetamine[7] and phenipraz-

†This work was supported in part by a Grant-in-Aid of the Society of the Sigma Xi and by the Research Institute of Pharmaceutical Sciences, University of Mississippi.

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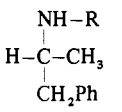
ine[8] resides in the *S* enantiomers. Pertinent to this observation are the reports that these three agents act at the same site of MAO.[9,10] A SAR study of 2-phenylcyclopropylamines has indicated that the major binding moieties of this class of MAO inhibitors are the phenyl and amino groups.[1] In this same study it was concluded that the degree of inhibitory activity of these compounds is related to the ability of the phenyl group to approach coplanarity with the C-2-C-3 atoms of the cyclopropane ring. A recent report[11] of the conformational analysis of amphetamine by nmr indicated a preferred trans disposition of the phenyl and amino groups. It is probable that pheniprazine exhibits a similar conformational preference although this has not yet been experimentally determined. Hence, the structural and stereochemical correlations found to exist for the MAO-inhibitory activity of tranylcypromine, amphetamine, and pheniprazine suggest a common mode of binding for these agents in their interactions with MAO. It is suggested that the phenyl and amino (hydrazino) groups primarily contribute to binding of the inhibitors to the site on the enzyme and that these moieties lie in distinctly different planes in the enzyme-inhibitor complex.



3a, R = H
3b, R = NH_2

It is of interest to note that similar pharmacophoric conformations have been proposed for tranylcypromine and amphetamine in their inhibition of the catecholamine uptake process in the CNS.[12] However, it is significant that the more active enantiomers of these agents as inhibitors of this process, (−)-2 and (+)-amphetamine, are of opposite stereochemistry at the common chiral center α to the amino group thereby suggesting dissimilarities in their modes of binding at the uptake process or perhaps different sites of action.

Studies of the steric aspects of the pharmacological actions of the cyclopropylamines are continuing in these laboratories.

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Antimalarial Activity of Clopidol, 3,5-Dichloro-2,6-dimethyl-4-pyridinol, and Its Esters, Carbonates, and Sulfonates†

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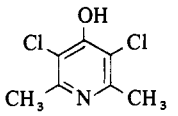
Received March 8, 1972

Clopidol,‡ 3,5-dichloro-2,6-dimethyl-4-pyridinol, **1**,[1] has been shown to possess antimalarial activity by the Walter Reed Army Institute of Research. It was active against the following strains of malaria: *Plasmodium berghei* in mice, *P. gallinaceum* in chicks, *P. cynomologi* in the *Macaca mulatta* monkey, and the *refractory* (chloroquine resistant) strain of *P. falciparum* in humans. It was considered a curative against *P. gallinaceum* at a dosage level of 160 mg/kg



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against *P. cynomologi* when given orally for 7 consecutive days. Studies with ^{36}Cl-labeled clopidol§ showed that clopidol is rapidly excreted from the body. Within 24 hr, 91% of the ^{36}Cl radioactivity was accounted for in the urine and feces of rats as unchanged clopidol.

Due to the rapid excretion of clopidol and its high insolubility in organic solvents as well as water, it was of interest to prepare derivatives of clopidol which would be more lipophilic and could in turn be hydrolyzed to clopidol *in vivo*. We have synthesized a series of esters, carbonates, and sulfonates of clopidol.

The esters and carbonates of clopidol, given in Table I, were prepared by treating the sodium salt of clopidol suspended in DMF with the corresponding acid chloride. It is of interest to note that the methyl and ethyl carbonates were prepared in high yields using this method even though ethyl chloroformate has been shown to have a half-life of only 9.5 min at 20° in DMF.[2] The sulfonates of clopidol, listed in Table II, were prepared by treatment of the sodium salt of clopidol with sulfonyl chlorides in a manner similar to the preparation of the esters and carbonates.

Antimalarial Data. Clopidol and its esters, carbonates, and sulfonates described herein have been tested for antimalarial activity against *P. berghei* in mice by Dr. Leo Rane at the University of Miami.# The results of these tests were furnished to us by Dr. E. A. Steck, Walter Reed Army Institute of Research. Three of the compounds were active: **1** at a dose level of 160 mg/kg, **9** at a level of 320 mg/kg, and **15** at 640 mg/kg. One may conclude that the greater lipophilicity of the derivatives of clopidol does not enhance the antimalarial activity. In as much as **9** and **15** were active, the synthesis of other hydrolyzable derivatives of clopidol is being carried out.

†This work was supported by the U. S. Medical Research and Development Command under the Contract No. DADA 17-69-C-9070. This paper is Contribution No. 1043 from the Army Research Program on Malaria.

§The ^{36}Cl-labeled clopidol was prepared by New England Nuclear Corp., Boston, Mass. 02118.

‡Clopidol is the active component of Coyden, a commercial coccidiostat sold by The Dow Chemical Co.

#For a description of test method see Osdene, *et al.*[3]