was taken up in the injection pipet (0.01 ml). In those instances in which there was insufficient residue, petroleum ether was added to wash traces into the pipet. The sample was injected, with the column temperature maintained at 200°C and with a helium flow rate of 40 ml/min and a chart speed of 5 in. per hour. These conditions do not represent the optimum for resolution but were selected to provide characteristic but short retention times to facilitate rapid analyses.

Table 1 shows the distribution of the hydrocarbons in the effluent from the silica-gel column, as determined by retention times on the gas column. It may be noted that, under the conditions employed, the silicic-acid column provided complete resolution. These results have been verified by a number of replications. In addition, larger samples (0.2 g) have been separated by the same procedure. A mixture (0.15 g) containing 80 percent limonene, 10 percent α-pinene, and 10 percent β-pinene was resolved, indicating that high concentrations of limonene did not interfere with resolution of the more rapid moving components of the mixture.

It is highly improbable that the above-mentioned conditions represent the optimum for separation of the hydrocarbons studied. Moreover, it is quite likely that any procedure will be dictated by the particular mixture to be resolved. It is significant, however, that the pinenes were separated, since they differ only in the position of the double bond. The pronounced retention of limonene is of special interest where the citrus oils are concerned because of the occurrence of high concentrations of this compound in these oils.

It should be possible to adapt the procedure to a preparative scale by employing columns of larger diameter. Upon elution of the hydrocarbons, oxygenated components may be separated by continuation with suitable solvents. In addition, the low temperature should inhibit the chemical changes often observed in adsorption chromatography. In conjunction with gas chromatography the technique may be especially useful, since the gas column can provide additional resolution.

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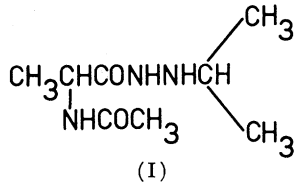
Stereospecificity of Monoamine Oxidase Inhibitors

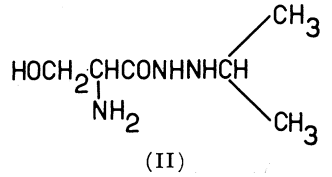
It has been found that isopropyl isonicotinic acid hydrazide (iproniazid, Marsilid) is a potent inhibitor of monoamine oxidase in vitro and in vivo. The alkylhydrazide group seems to be the active principle. Isonicotinic acid hydrazide (isoniazid, Rimifon), a compound similar to iproniazid but not containing the isopropyl group, is only a weak monoamine oxidase inhibitor. Furthermore, according to previous workers and to our own findings, N-isopropylhydrazine inhibits monoamine oxidase more markedly than iproniazid does (*1*).

In order to decrease toxicity and to increase specificity of monoamine oxidase inhibitors in vivo, isopropylhydrazides of substances which occur naturally in the body—for example, the amino acids alanine (acetate)



(I)

and serine



(II)

were synthesized (*2*). It was observed that the derivatives of the *l*-amino acids behaved differently from those of the unnatural *d* isomers.

After administration of the *l* forms of I and II, a considerable increase in 5-hydroxytryptamine (5HT, serotonin) content occurred in the brain of rats, as measured by a spectrophotofluorometric method (*3*). The rise was much more marked than after equimolecular doses of iproniazid. The *d* forms of I and II had significantly less effect on the 5-hydroxytryptamine content of brain than the *l* forms ($p < 0.01$). In fact, with the *d* form of I, no significant increase in 5-hydroxytryptamine could be observed at all (see Table 1).

In vitro, there was also a significant difference between the *d*- and *l*-amino acid hydrazides ($p < 0.01$). However, no correlation could be found between the monoamine oxidase inhibition in vivo (as measured by the rise in 5-hydroxytryptamine in the brain) and in vitro (as measured in mitochondrial suspensions and supernatant). In fact, the *l* forms of I (*4*) and II showed markedly more activity in vivo than iproniazid did. In in vitro studies this was not the case. Thus, the *l* form of I caused significantly less monoamine oxidase inhibition in mitochondrial suspensions and liver supernatant than iproniazid ($p < 0.01$) did. The difference in activity between the *l* form of II and iproniazid was not significant ($p > 0.05$).

These results show that the steric configuration of the acyl moiety is an essential factor in the activity of the above-mentioned isopropylhydrazides on monoamine oxidase in vivo and in vitro. A similar stereochemical difference has been found for amino acid hydrazides without substitution in N_2, with respect to action on diamine oxidase preparations (*5*). This stereospecificity may be partly due to the fact that both amine oxidases contain an optically active center, too. The higher relative activity of isopropylhydrazides of natural amino acids, especially *l*-alanine, in vivo as compared to in vitro (see Table 1), is probably due to an additional factor. It

Table 1. Monoamine oxidase inhibition by amino acid hydrazides as compared with iproniazid.

Inhibitor	Rise of 5HT in rat brain (%) in vivo;* (iproniazid = 100 ±10)	Inhibition of monoamine oxidase (%) in vitro;† (iproniazid = 100 ± 10)	
		Mitochondria (rat brain)	Supernatant (guinea pig liver)
l-I	195 ± 10	55 ± 6	67 ± 11
d-I	6 ± 4	15 ± 8	15 ± 11
l-II	200 ± 9	142 ± 20	106 ± 12
d-II	144 ± 15	80 ± 14	76 ± 8

* Iproniazid in the amount of 100 mg/kg was injected intraperitoneally 16 hr prior to 5HT determination. † Brain and liver were homogenized in 0.25*M* sucrose. The mitochondria were isolated by centrifugation at 500*g* for 5 minutes and subsequently at 23,000*g* for 15 minutes, resuspended in 0.06*M* phosphate buffer (*p*H 7.3), and frozen. The supernatant was diluted 1/1 with 0.1*M* phosphate buffer (*p*H 7.3). The brain mitochondria as well as the liver supernatant were aged for 1½ hours at 37°C. Oxygen consumption was measured, tyramine (3×10^{-3} mole/liter) being used as substrate. Concentration of the inhibitors in the supernatant and of the mitochondrial suspensions were 5×10^{-5} and $3.3 \times ^{-5}$ mole/liter, respectively. At this concentration iproniazid caused monoamine oxidase inhibition of about 50 percent. The enzyme preparations were preincubated with the inhibitors for 15 minutes prior to dipping in the substrate.

is conceivable that naturally occurring substances (for example, *l*-amino acids) have transport mechanisms available in the body which are not present for unnatural compounds. Thus, *l*-amino acids might serve as carriers for the active isopropylhydrazide moiety through natural barriers (such as the blood-brain barrier).

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Estimation of Total Body Fat from Roentgenograms

Measurements of the thickness of the subcutaneous fat can be used for estimating man's fatness, and percentile norms for judging the relative fatness of individuals in this way have been provided (*1*). For some purposes, however, an estimate of body fat is desired in absolute rather than in relative terms. Such an absolute estimate can be made with the rather complicated methods of body water and body density measurements. This estimate, in turn, can be used to establish predictions for total fat based on the simpler measurements of subcutaneous fat (*2*). This procedure has proved satisfactory in obtaining estimating equations for total fat from skinfold measurements in young and middle-aged men (*3, 4*).

In the study described in this report the thickness of subcutaneous adipose tissue (plus skin) was measured on soft-tissue roentgenograms, taken at a distance of 72 in. between the tube and the film. No correction for triangular distortion was applied. Data are reported for four sites: (i) upper arm, at the level of the deltoid insertion (see *5*); (ii) upper arm, one-third of the distance between olecranon process and acromion; (iii) forearm; and (iv) calf, at the level of maximal width. All projections were anteroposterior. At site No. 1 the measurements were made vertically to the skin, and at the other three sites they were made vertically to the long axis of the limb. At sites No. 2, 3, and 4 both the lateral and medial thicknesses were measured and summated. Total body fat was estimated from body density, the body volume being obtained by underwater weighing with individual corrections for air remaining in the lungs and respiratory passages at the moment weight was recorded.

Middle-aged business men and professional men, participating in a longitudinal study of aging (*6*), were the subjects. The analysis was restricted to 52 men (mean age 57.1, S.D. = ± 2.7 years) whose weight did not change by more than ± 2 percent from the time of the density measurements to the time when the roentgenographic data were obtained 4 years later.

Equations for predicting body density from roentgenographic measurements and the coefficients of correlation between the two types of criteria of leanness-fatness are given in Table 1. The correlations here recorded must be considered to be slightly depressed from those that would be obtained with measurement of total fat and recording of the x-ray patterns on the same day. But the correlations in Table 1 are in the same general range as those reported when subcutaneous fat was measured with skinfold calipers (*3*). No precise comparison between the x-ray and the skinfold caliper measurements previously made is possible because of differences in the measurement sites.

The number of individuals for whom satisfactory x-ray data were available in all four sites was relatively small. Consequently, no attempt was made to relate body density to roentgenographic measurements in the form of a multiple-regression equation. This is a task for further research in which attention should be given also to some areas on the trunk, including those not readily measurable by skinfold calipers, such as the trochanteric area.

Table 1. Equations ($\hat{D} = a + bX$) for predicting body density from roentgenographic measurements (X, in millimeters) and coefficients of correlation (r) between density and roentgenographic measurements. N = size of the sample.

Site	a (intercept)	b (slope)	r	N
1. Deltoid insertion	1.07220	−0.00186	−.60	42
2. Upper arm	1.07812	−0.00219	−.75	41
3. Forearm	1.07294	−0.00309	−.76	51
4. Calf	1.06447	−0.00228	−.58	47

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Effect of Reserpine Pretreatment on Stimulation of the Accelerans Nerve of the Dog

Abstract. Pretreatment with two doses of reserpine (each 0.1 mg/kg, intraperitoneally) sensitizes the heart to the positive chronotropic action of norepinephrine and reduces the response to stimulation of the accelerans nerve. Ganglionic transmission remains unaffected. The results indicate that the presence of certain stores of peripheral sympathetic transmitter is essential for the production of tachycardia by stimulation of the accelerans nerve.

Recent experiments (*1*) show that reserpine is capable of causing a tachycardia in the heart-lung preparation of the dog by liberation of norepinephrine from its stores in the heart. Pretreatment of the dogs with reserpine prior to the isolation of the heart, by depletion of the stores of norepinephrine, prevented the positive chronotropic response of the heart-lung preparation to the challenging dose of reserpine. A dose of 0.1 mg/kg injected intraperitoneally 24 hours before the heart-lung preparation was set up was found to suffice for the pretreatment (*2*).

Other experimental evidence indicates that pretreatment with reserpine abolishes the stimulant action of nicotine on isolated rabbit atria (*3*); in this preparation nicotine acts presumably on ganglion cells or chromaffine tissue situated in the heart wall, or on both, and thus liberates sympathin. Reserpine has also been found to reduce the norepinephrine content of sympathetic ganglion cells (*4*).

The experiments described in this report (*5*) were undertaken in order to