response which progressed throughout the entire tissue exposure time. If given simultaneously with either CH-20 or TH-27, the onset of the negative inotropic effect was delayed by the calcium.

SUMMARY AND CONCLUSIONS

As a group, the nonethynyl cyclohexanol derivatives were 2 to 7 times more potent than were the derivatives of cyclohexylamine in producing a hypotensive response in the intact rat. *trans*-2-*o*-Tolyl-*cis*-1,4-cyclohexanediol (CH-14) and *trans*-2-*o*-tolyl-*trans*-1,5-cyclohexanediol (CH-20) were equally effective and the most active, while the *trans* isomer of the tertiary cyclohexylamine (TH-25) was the least active and the only compound that produced a pressor response. The cyclohexylamine derivatives were up to 8 times more potent, in arresting the isolated guinea pig heart, than were the derivatives of cyclohexanol. The *trans* and *cis* isomers of the tertiary cyclohexylamine (TH-25 and TH-27) were equally effective and the most active; while *trans*-2-*o*-tolyl-*cis*-1,4-cyclohexanediol (CH-14) possessed the least amount of cardio-inhibitory activity among those compounds studied. An increase in the number of hydroxyl substituents on the cyclohexanol ring was accompanied by a decrease in both hypotensive and cardio-inhibitory activity. The minimum effective dose of 1-ethynyl-*trans*-2-*o*-tolylcyclohexanol (AH-64) in producing cardiac arrest was also the cardiotoxic dose; while the *cis* isomer (AH-65) showed the greatest cardio-inhibitory activity among the cyclohexanol derivatives studied. Spatial configuration was also significant in the blood pressure activity of the tertiary cyclohexylamines. The *cis* isomer (TH-27) produced a depressor response in contrast to the pressor response elicited by the *trans* isomer (TH-25). In the isolated atrial preparation, calcium antagonized the negative inotropic effects of *trans*-2-*o*-tolyl-*trans*-1,5-cyclohexanediol (CH-20) and *cis*-2-(*p*-chlorophenyl)-*N,N*-dimethylcyclohexylamine hydrochloride (TH-27). Moreover, calcium momentarily restored contraction of the heart arrested by 1-ethynyl-*cis*-2-*o*-tolylcyclohexanol (AH-65). In addition to its negative inotropic effect on the isolated atria, *cis*-2-(*p*-chlorophenyl)-*N,N*-dimethylcyclohexylamine hydrochloride (TH-27) also produced a negative chronotropic response which was unaffected by the administration of calcium. Decreased contraction of the myocardium appears to be responsible for the cardioplegia produced by the cyclohexanol compounds, since the pacemaker activity of the isolated atrial preparation is not affected. In addition to a decreased contraction of the myocardium, alteration of impulse formation or suppression of impulse conduction appear to be responsible for the cardioplegia produced by the cyclohexylamine derivatives. Preliminary studies of the pressor response to *trans*-2-(*p*-chlorophenyl)-*N,N*-dimethylcyclohexylamine hydrochloride (TH-25) indicate that the compound is capable of producing direct peripheral vasoconstriction.

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Correlation of Dissolution Rate and Griseofulvin Absorption in Man

By B. KATCHEN and S. SYMCHOWICZ

Five different griseofulvin preparations were studied in 10 healthy subjects. Plasma levels were followed for 24 hr. after single oral 500-mg. doses. The dissolution rate of each dosage form was measured in simulated intestinal fluid and distilled water. Good correlation was seen between dissolution rates in simulated intestinal fluid and griseofulvin absorption in man.

GRISEOFULVIN is given in large amounts and is insoluble in water. Its dose in man is 125–500 mg., and its water solubility is 15 mcg./ml. at 37°. If the digestive fluids are saturated with respect to griseofulvin at all times, and griseofulvin solubility in digestive fluids is the same as in distilled water, a minimum of 33 L. of digestive fluids must be cleared for complete absorption of 500 mg. of griseofulvin as shown by the following calculation: dose (500 mg.)/solubility (15 mg./L.) = 33 L. Clearly, oral griseofulvin dosage

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TABLE I—DISSOLUTION RATES OF GRISEOFULVIN PREPARATIONS

Dissolution Medium	Time, min.	% Dissolved Prepn. 1	Prepn. 2	Prepn. 3	Prepn. 4
Distilled water	5	4	4	0	0
	10	6	6	2	0
	15	9	7	5	34
	30	13	11	10	93
	45	18	13		100
	60	22	16	15	
Simulated intestinal fluid	5	6	7	3	0
	10	9	15	12	27
	15	11	24	26	69
	30	13	35	56	96
	45		45		100
	60		52	75	

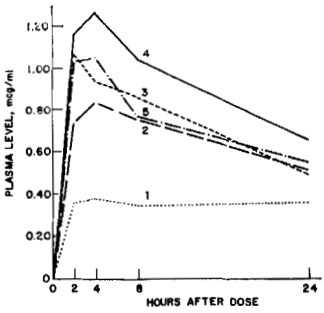

Fig. 1—Griseofulvin plasma levels. Plasma samples drawn at 0, 2, 4, 8, and 24 hr. postdrug. Each point is the mean of 10 values. Numeral near each curve is the preparation number.

TABLE II—GRISEOFULVIN PLASMA LEVELS (mcg./ml.) IN MAN FOLLOWING AN ORAL 500-mg. DOSE

Prepn.	Time, hr.	Subject[a] A	B	C	D	E	F	G	H	I	J	Mean	Area/24
1	2	(0.39)[b]	0.24	0.24	0.21	0.63	0.31	0.45	0.41	0.47	0.23	0.35	0.34
	4	(0.36)	0.25	0.31	0.10	0.76	0.27	0.64	0.46	0.44	0.17	0.38	
	8	(0.32)	0.24	0.34	0.20	0.55	0.21	0.68	0.33	0.33	0.21	0.34	
	24	(0.66)	0.32	0.33	0.55	0.38	0.27	0.37	0.47	0.22	0.32	0.36	
2	2	0.60	0.20	0.72	0.28	2.12	0.72	0.36	0.88	1.00	0.50	0.74	0.65
	4	0.60	0.24	0.59	0.66	1.80	0.78	1.67	0.70	0.86	0.47	0.84	
	8	0.71	0.80	0.59	0.21	1.44	0.74	1.45	0.50	0.69	0.36	0.75	
	24	0.83	0.61	0.59	0.19	0.57	0.48	0.48	0.45	0.50	0.43	0.51	
3	2	0.93	(0.62)	0.64	1.03	1.50	1.18	1.05	1.20	1.05	1.05	1.07	0.73
	4	1.07	(0.54)	0.47	1.00	1.22	0.96	0.95	0.78	0.94	1.11	0.94	
	8	0.88	(0.68)	0.36	0.90	1.47	0.76	0.81	0.55	1.13	0.86	0.86	
	24	0.76	(0.46)	0.28	0.46	0.55	0.58	0.20	0.35	0.62	0.59	0.49	
4	2	1.41	0.64	1.12	1.05	1.60	1.21	0.86	1.31	1.19	1.23	1.16	0.90
	4	1.01	1.00	0.90	1.61	1.40	1.26	1.90	0.93	0.48	1.10	1.26	
	8	1.00	0.71	0.79	1.20	1.60	0.80	1.90	0.64	0.91	0.83	1.04	
	24	0.99	0.45	0.64	0.79	0.80	0.61	0.62	0.44	0.54	0.61	0.65	
5	2	0.79	0.77	0.82	0.76	1.77	1.20	0.98	0.92	1.42	0.88	1.03	0.72
	4	0.79	0.34	0.82	0.78	0.94	1.10	1.17	1.03	1.40	1.01	1.05	
	8	0.44	0.44	0.62	0.63	1.57	0.52	0.98	0.71	1.11	0.64	0.77	
	24	0.69	0.69	0.42	0.40	0.64	0.39	0.45	0.45	0.60	0.81	0.56	

[a] Subjects A–E treated in hospital *A*; F–J, in hospital *B*. [b] Figures in parentheses used in place of missing results; values estimated *via* method of unweighted means.

forms must be carefully designed and evaluated to insure maximum drug availability.

Several factors affecting griseofulvin plasma levels were studied (1–6) but the effect of dissolution rate on griseofulvin absorption was not measured. This study will correlate dissolution rate of griseofulvin dosage forms and griseofulvin absorption in man.

EXPERIMENTAL

Materials—Five different 125-mg. griseofulvin preparations were studied.

Methods—At 1-week intervals, 5 different griseofulvin preparations were given to 10 humans in a three-factor repeat measure design (7). Each subject took 500 mg. griseofulvin before breakfast at approximately 8:00 a.m. and ate a standard meal at 10:00 a.m. The subjects were told to avoid, for the next 24 hr., the foods and drugs which interfere with the griseofulvin plasma assay (8).

Blood was drawn at 0, 2, 4, 8, and 24 hr. postdrug and the plasma samples were assayed fluorometrically for griseofulvin (9). All plasma levels were corrected for their zero-hour values.

Mean plasma level, which was the index of absorption, was calculated by dividing the area (determined by the trapezoidal rule) under the 24-hr. plasma level curve by 24.

Dissolution rates of single 125-mg. tablets or capsules were measured (10) in either 20 L. of distilled water or 20 L. of simulated intestinal fluid (dissolve 20 Gm. of pH 7.2 phosphate buffer powder[1] and an aqueous extract of 5 Gm. of enzyme powder[2] in 20 L. of distilled water).

RESULTS AND DISCUSSION

Dissolution Rates—Table I shows the dissolution rate of four preparations in distilled water and simulated intestinal fluid. Preparation 5 could not be analyzed because its contents clogged the 40-mesh screen of the sample holder; therefore, its

[1] Hartman-Leddon Co., Philadelphia, Pa.
[2] Marketed as VioKase by the VioBin Corp., Monticello, Ill.

TABLE III—ANALYSIS OF VARIANCE OF GRISEOFULVIN PLASMA LEVELS

Source	d.f.	*MS*	*F*	*p*
Between subjects	9			
Hospital	1	.0024	<1	>0.27
Subjects within hospital	8	.8722		
Within subjects	190			
Treatment	4	2.2643	21.77	<0.01
Treatment × hospital	4	0.0321	<1	>0.25
Treatment × subject within hospital	32	0.1040		
Hour	3	1.3310	8.20	<0.01
Hour × hospital	3	0.0621	<1	>0.25
Hour × subject within hospital	24	0.1624		
Treatment × hour	12	0.0137	4.22	<0.01
Treatment × hour × hospital	12	0.0082	<1	>0.25
Treatment × hour × subject	96	0.0324		

TABLE IV—ANALYSIS OF GRISEOFULVIN MEAN PLASMA LEVELS IN MAN BY DUNCAN'S MULTIPLE RANGE STATISTIC

Treatment	Area/24
1	0.34
2	0.65
5	0.72
3	0.73
4	0.90

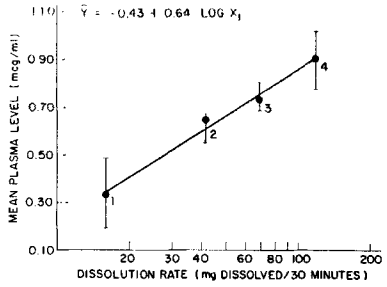

Fig. 2—Correlation of dissolution rate and mean griseofulvin plasma level. Correlation coefficient = 0.995 (p = 0.02). Brackets enclose 95% confidence intervals of regression line. Numeral beneath each point is the preparation number.

dissolution rate could not be correlated with absorption of griseofulvin.

In distilled water (Table I) the dissolution rates of preparations *1*, *2*, and *3* are very low and cannot be differentiated. However, in simulated intestinal fluid, except for preparation *1*, the dissolution rates are much higher and reveal differences between preparations related to their formulations. Thus, intestinal fluid is a more sensitive tool for evaluation of griseofulvin formulations.

Plasma Levels—The individual assay data are shown in Table II. Peak plasma levels, which vary from 0.35 to 1.26 mcg./ml., occur in 2 to 4 hr. (Fig. 1) and then decline slowly for the next 20–22 hr. at varying rates. Preparations *3* and *4*, which have the highest dissolution rates in simulated intestinal fluid, decline more rapidly than preparations *1* and *2*, which have the lowest dissolution rates in simulated intestinal fluid.

The sustained low plasma level of preparation *1* suggests slow and prolonged drug release *in vivo*. In contrast, the much higher peak plasma levels and disappearance rates of preparations *3* and *4* suggest early and rapid drug release *in vivo*. An analysis of variance procedure for repeat measure designs (Table III), which assumes a fixed effects model, was applied to the plasma levels.

The analysis shows treatments, time, and the treatment × time interaction are significant factors ($p < 0.01$). Duncan's multiple range test was applied to the area/24 values (Table IV) which were calculated for each set of plasma levels in Table II. The values not connected by a common line differ significantly ($p < 0.05$).

Correlation of Dissolution Rate and Griseofulvin Absorption—Because each preparation could not be characterized with a single dissolution rate constant, the authors arbitrarily selected amount dissolved in a given time as a measure of dissolution rate. A meaningful correlation between mean plasma level and dissolution rates in distilled water could not be found because most of the griseofulvin preparations have low dissolution rates in this solvent. However, a good correlation was found between mean griseofulvin plasma level and dissolution rate in simulated intestinal fluid. The best correlation is obtained with the logarithm of the 30-min. dissolution values (Fig. 2). The correlation coefficient is 0.995 ($p = 0.01$). The 15-min. dissolution values have a correlation coefficient of 0.96 ($p = 0.05$); the 10-min. values have a correlation coefficient of 0.82 ($p = 0.10$).

The high correlation coefficients in this study were obtained with preparations that differ widely in their composition and dissolution rates. This suggests that the ability of various griseofulvin formulations to increase plasma drug levels in man (1–3) might have been due to improved dissolution rates.

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Conformational Analysis of 4-Hydroxycyclohexanone Oxime by NMR Spectroscopy

By WILLIAM F. TRAGER and ALAIN C. HUITRIC

The conformational free energy of the hydroxyl group of 4-hydroxycyclohexanone oxime has been found to be 0.33 and 0.61 Kcal./mole in pyridine and D_2O, respectively. These values are intermediate to those of the hydroxyl group of cyclohexanol and 4-hydroxycyclohexanone in the corresponding solvents. Comparison of the spectrum of 4-*tert*-butylcyclohexanone-3$_{(axial)}$,5,5-d_3 oxime with that of 4-hydroxycyclohexanone oxime and utilization of the long-range deshielding effect of the oximido group has permitted assignment of the individual resonances in the spectrum of the latter compound to specific hydrogens. Moreover, the comparison of spectra and use of the oximido group long-range deshielding effect has provided an independent check on the validity of the equilibrium positions calculated herein.

THE CONFORMATIONAL free energy of the hydroxyl group ($-\Delta G°_{OH}$) in cyclohexane systems has been the subject of extensive investigations over the past 10 years and is now well established. The results are summarized by Eliel and Schroeter (1). Values of 1.25 Kcal./mole in D_2O (2) and 0.83 in pyridine (3) have been obtained for cyclohexanol. It is only recently that comparative studies have been extended to six-membered saturated ring systems containing a hetero atom (4) or a trigonal carbon (5). Since the authors have found a significant difference in the values of $-\Delta G°_{OH}$ for cyclohexanol and 4-hydroxycyclohexanone at similar concentrations in a given solvent (5)[1] it was of interest to extend the investigation to the other six-membered ring compounds containing a trigonal carbon atom. The observation of an unusually large chemical shift difference between the geminal hydrogens adjacent to the oximido group in 4-*tert*-butylcyclohexanone oxime (6) pointed to 4-hydroxycyclohexanone oxime as an attractive system to study both phenomena.

DISCUSSION

Conformational Analysis—Conformational preference of the two chair conformers Ia and Ib, Fig. 1, was determined by the signal width method (5, 7–12) as described for 4-hydroxycyclohexanone (5). The mole fraction of conformer Ia (*Na*) was obtained from w, the width of the signal of H-4 (hydrogen Z) in the mobile system, and from w_e and w_a, the widths of the signals of H-1 in the two conformationally homogeneous models *trans*- and *cis*-4-*tert*-butylcyclohexanol-3$_{(axial)}$,5,5-d_3, II and III, respectively, by the following relationship (5):

$$Na = \frac{w - w_a}{w_e - w_a}$$

From the spectrum of II in pyridine, w_e was found to be 30.0 c.p.s., Jaa = 10.7, Jae = 4.4, and J_{gem} = 12.0 c.p.s.; and the spectrum of III in pyridine gave w_a = 11.0 c.p.s., Jea $\simeq$ Jee $\simeq$ 2.7 c.p.s. (5).

In pyridine the signal of Z has a width w, of 23 c.p.s. which leads to a value of 0.63 for the mole fraction *Na* of conformer Ia and a free energy difference, $-\Delta G°_{OH}$, of 0.33 Kcal./mole at 37°. In D_2O[2] the width of the signal measured from spectrum E is 24.8 c.p.s., leading to an *Na* value of 0.73 and $-\Delta G°_{OH}$ of 0.61 Kcal./mole at 37°. The addition of about two equivalents of diethylamine in pyridine did not cause any change in the width of the signal of Z. Addition of slightly over one equivalent of NaOH in D_2O caused an increase of about 0.5 c.p.s. in w. This suggests that there is very little, if any, ionization of the oxime in pyridine under the conditions of the measurements.

Assignment of Signals to Specific Hydrogens—The assignment of the signal of the Z hydrogen is obvious in all spectra. In the 60 Mc. spectra it occurs at τ 6.05 in D_2O and τ 5.91 in pyridine. In the 100 Mc. spectrum in pyridine it occurs at τ 5.88. There is also a slight difference in the chemical shift of the X hydrogen obtained from the 60 and 100 Mc. spectra in pyridine: τ 6.73 at 60 Mc. and

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[1] 0.28 Kcal./mole in D_2O and zero in pyridine for 4-hydroxycyclohexanone.

[2] The model compounds II and III are not sufficiently soluble for measurements in D_2O, and the w_e and w_a values obtained in pyridine were used. The danger of error due to variation of coupling constants with solvent in these rigid systems seems minimal because of the observation that the difference in the width of the signal of H-1 in 4-*tert*-butyl-*cis*-4-hydroxycyclohexanol-3,3,5,5-d_4 (13) measured in pyridine and 25% acetic acid in D_2O is only 0.5 c.p.s.