220–221°, and weighing 17.9 mg., lit. m.p. for aloe-emodin 223–225° (5). TLC in several different solvent systems showed the isolate to be homogeneous and identical in R_f value with reference aloe-emodin.[3] IR spectra of the isolate and reference aloe-emodin were superimposable, and the UV absorption spectrum for the isolate was λ_{max}^{EtOH} 226, 253, and 287 mμ (log ε 4.2, 3.8, and 3.5).

SUMMARY

A phytochemical investigation of *Cassia reticulata* flowers has resulted in the isolation of rhein, aloe-emodin, and β-sitosterol, which have not been previously reported from the flowers of this plant. At least one additional anthraquinone was observed to be present, in addition to three flavonoids, but these were not isolable using the methods employed in this study.

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[3] A reference sample of aloe-emodin was provided by Professor J. W. Fairbairn.

Keyphrases

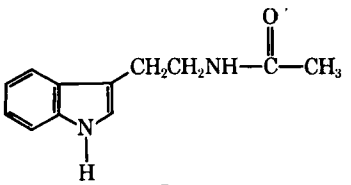
Cassia reticulata flowers—phytochemistry
Anthraquinones—isolated, identified
β-Sitosterol—isolated, identified
Column chromatography—separation
TLC—identity
IR spectrophotometry—identity
UV spectrophotometry—identity

Hydroxyindole-*O*-methyltransferase II. Inhibitory Activities of Some *N*-Acyltryptamines

By BENG T. HO, WILLIAM M. McISAAC, L. WAYNE TANSEY, and PATRICIA M. KRALIK

Several *N*-acyltryptamines were found to inhibit hydroxyindole-*O*-methyltransferase. Of these inhibitors the most active was *N*-phenylacetyltryptamine, having an affinity to the enzyme six times greater than the substrate *N*-acetylserotonin. These studies showed that the alkyl and aralkyl groups of the amides were most likely complexed with the enzyme by hydrophobic bonding.

In the previous paper (1), the binding of the substrate *N*-acetylserotonin to the enzyme hydroxyindole-*O*-methyltransferase (HIOMT) was studied. There was a good indication that the CH_3 of the amide I was complexed with the enzyme by



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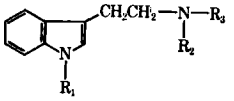
hydrophobic bonding. To explore this hydrophobic region on HIOMT, several substituted *N*-acyltryptamines (II–VIII) were synthesized and their inhibitory activities evaluated (Table I).

The finding that both *N*-cyclobutylcarbonyl-

TABLE I—INHIBITION OF HIOMT



Compound	R_1	R_2	R_3	I_{50},[a] (m*M*)
I	H	H	$COCH_3$	1.40[b]
II	H	H	COC_4H_7	0.88
III	H	H	COC_6H_{11}	0.85
IV	H	H	COC_6H_5	0.37
V	H	H	$COCH_2C_6H_5$	0.18
VI	H	H	$COCH_2CH_2C_6H_5$	0.22
VII	H	CH_3	$COCH_2C_6H_5$	0.16
VIII	CH_3	H	$COCH_2C_6H_5$	0.35
IX	H	H	$SO_2C_6H_5$	0.37

[a] Concentration of an inhibitor giving 50% inhibition of the enzyme. [b] Data from the previous paper (1).

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TABLE II—PHYSICAL CONSTANTS OF *N*-ACYLTRYPTAMINES

Compound	Method[a]	Yield, %	M.p.,[b] °C.	Anal. Calcd.	Anal. Found
II	A	67	110–111[c]	C, 74.4	74.3
				H, 7.49	7.48
				N, 11.6	11.5
III	A	63	105–106[c]	C, 75.5	75.4
				H, 8.20	8.19
				N, 10.4	10.3
IV[d]	B	39	130–132[e]		
V[f]	A	54	144–145[g]		
VI	B	88	74–75[c]	C, 78.0	77.9
				H, 6.90	6.99
				N, 9.58	9.63
VII	A	66	104–105	C, 78.0	78.3
				H, 6.90	7.10
				N, 9.58	9.47
VIII	B	79	76–76.5[c]	C, 78.0	77.9
				H, 6.90	6.81
				N, 9.58	9.47
IX	B	90	88.5–89.5[h]	C, 64.0	64.0
				H, 5.37	5.58
				N, 9.33	9.21

[a] See *Experimental*. [b] Melting points were taken on a Fisher-Johns apparatus and are corrected. [c] Recrystallized from aqueous ethanol. [d] Lit. (3) m.p. 137–138° (benzene). [e] Recrystallized from benzene. [f] A melting point of 142–143.5° (benzene) has been recorded for this compound prepared by the reaction of tryptamine with phenylacetic acid (3). [g] Recrystallized from ethanol. [h] Recrystallized from benzene–hexane.

tryptamine (II) and *N*-cyclohexylcarbonyltryptamine (III) were better inhibitors of HIOMT than I seemed to support the existence of a hydrophobic interaction of the cyclobutyl and cyclohexyl groups with the enzyme. A further increase in inhibitory activity was observed when the methyl group of I was replaced by a phenyl group. The resulting compound, *N*-benzoyltryptamine (IV) was nearly four times more active than I. This increase in activity was attributed to the greater affinity of the phenyl ring to the enzyme as compared to the methyl group. A phenyl ring is capable of complexing with the enzyme by both hydrophobic bonding and donor–acceptor interaction, whereas an alkyl group could only bind to the enzyme by hydrophobic inaction. This could account for the greater than twofold difference in activity between IV and II or III. Insertion of a methylene group between the phenyl and C=O of IV resulted in an inhibitory activity eight times greater than I (see V). It was observed that a maximum binding could be obtained with the $C_6H_5CH_2$ of V, since extension of aralkyl chain from $C_6H_5CH_2$ to $C_6H_5CH_2CH_2$ (VI) did not give further increase in inhibitory activity.

Substitution of the amide hydrogen of V by a methyl group (see VII) did not cause any change in the activity of V. This finding agreed well with the previous finding (1). Methylation of the indole nitrogen of V, however, resulted in a twofold decrease in activity (see VIII). Whether this decrease was due to the loss of the indolic hydrogen, which otherwise would form a hydrogen bond with the enzyme, or whether the 1-methyl group caused steric hindrance to the binding of the indole nucleus to the enzyme remains to be investigated.

Compound IX which has a structural resemblance to IV was found to be equally as active as IV.

Kinetics studies showed that V was bound to the enzyme six times and IV four times stronger than the substrate *N*-acetylserotonin, with $Km = 5.7 \times 10^{-5}$ *M*, Ki (V) $= 0.92 \times 10^{-5}$ *M*, and Ki (IV) $= 2.8 \times 10^{-5}$ *M* obtained by the reciprocal plot method. They were competitive inhibitors of the enzyme.

EXPERIMENTAL

Synthesis—*Method A*—To a solution of 10 mmoles of tryptamine or methyltryptamine in 25 ml. of chloroform was added 10 mmoles of triethylamine and an equivalent amount of acid chloride in chloroform. The mixture was stirred at room temperature for 30 min. The chloroform layer was separated, washed successively with 50 ml. of 10% HCl, 50 ml. of 2*N* NaOH, and 50 ml. of water, dried with anhydrous sodium sulfate, and evaporated *in vacuo*. In the cases of III and VII, the residue was an oil which solidified upon standing or drying in a desiccator *in vacuo*.

In the preparation of V, instead of chloroform 100 ml. of benzene was used. The product, which precipitated during a 30-min. period, was collected on a filter and washed successively with acid, base, and water.

See Table II for compounds prepared by this method and their physical constants.

Method B—A solution of 10 mmoles of tryptamine hydrochloride or 1-methyltryptamine hydrochloride (2) in 25 ml. of water was neutralized with 15 ml. of 10% aqueous sodium hydroxide. Dichloromethane (50 ml.) was added to dissolve the precipitate, followed by an addition of 10 mmoles of acid chloride. The mixture was stirred at room temperature for 1 hr. The organic layer was washed successively with 10% NaOH, 10% HCl, and water, and dried with anhydrous sodium sulfate. Evaporation of solvent under reduced pressure gave an oily residue which solidified upon standing.

In the preparations of IV and IX the organic solvent phase was omitted.

See Table II for compounds prepared by this method and their physical constants.

Assay—Hydroxyindole-*O*-methyltransferase was

isolated from beef pineal gland and purified according to the method of Axelrod and Weissbach (4).

The stock solutions of inhibitors II, III, IV, and VII were prepared in propylene glycol, and those of V, VI, VIII, and IX were prepared in dimethylsulfoxide. The previous findings showed that the same magnitude of inhibitory activity was obtained regardless of the use of these two solvents (1).

Incubation was carried out with *N*-acetylserotonin and *S*-adenosyl-L-methionine-methyl-^{14}C according to the previously described procedure (1).

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Keyphrases

Enzyme inhibitors
N-Acetyltryptamines—synthesis
Hydroxyindole-*O*-methyltransferase inhibition—*N*-acyltryptamines

Effect of Flow Rate on the Distribution Kinetics of a Drug From Perfusate to a Perfused Organ

By RENPEI NAGASHIMA and GERHARD LEVY*

A pharmacokinetic analysis of the distribution of a drug into a perfused organ or hypothetical compartment of the body yields mathematical expressions which may be useful for ascertaining if the distribution of a drug is rate limited by the flow of the perfusate, by the diffusion of the drug from the perfusate into the organ, or if it is a function of both of these processes. These expressions have been used to analyze the distribution of a drug from a perfusing fluid into the isolated perfused rat liver, and of several drugs from a hypothetical central compartment to a hypothetical peripheral compartment in man.

THE DISTRIBUTION of a drug from a perfusion fluid such as the blood to a perfused organ such as the liver is likely to be rate limited by the flow of the perfusate, by the diffusion of drug from perfusate to the organ, or it may be a function of both of these processes. It is useful therefore to undertake a kinetic analysis of such a system so that the rate-limiting step in the distribution of a given drug under defined conditions may be determined.

For the purpose of this analysis, the liver is viewed as a tissue with numerous parallel channels through which perfusate flows. The concentration of the drug in the perfusate leaving the liver is lower than the drug concentration in the fluid entering the liver, due to the diffusion of drug from perfusate into the liver. Elimination of the drug by the liver and back-diffusion of the drug from the liver to the perfusate are considered to be negligible during the early distribution phase. These assumptions lead to the same model as has been presented in an earlier report from this laboratory dealing with drug elimination kinetics in a perfused organ (1). Equation 8 of that report then takes the form:

$$k_{\text{dist.}} = \frac{u}{V_R}[1 - \exp(-k_{\text{trans.}}V_L/u)] \quad \text{(Eq. 1)}$$

where $k_{\text{dist.}}$ is the apparent first-order rate constant for the distribution of drug from perfusate to the perfused organ or tissue, u is the flow rate of perfusate, V_R is the real or apparent volume of the extrahepatic perfusate (which in practice usually is equivalent to the real or apparent volume of total perfusate), V_L is the volume of the channels in the organ or tissue through which the perfusate flows, and $k_{\text{trans.}}$ is a rate constant for the transfer of the drug from perfusate to the organ or tissue.[1] When drug distribution is rate limited by the flow of the perfusate, *i.e.*, $u \ll k_{\text{trans.}}V_L$, the bracketed term in Eq. 1 approaches unity, and

$$k_{\text{dist.}} = \frac{u}{V_R} \quad \text{(Eq. 2)}$$

If, on the other hand, the distribution of the drug is rate limited by the transfer of the drug from perfusate to the tissue, $k_{\text{trans.}}V_L/u < 0.1$ and it can be shown (1) that

$$k_{\text{dist.}} = \frac{V_L}{V_R} \cdot k_{\text{trans.}} \quad \text{(Eq. 3)}$$

It should be noted that Eq. 3 does not contain the flow-rate term.

One may now turn to a consideration of the more general case, namely, the distribution and elimination of the drug during the entire experimental

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[1] This transfer rate constant may be the rate constant for diffusion of the drug through the blood, through a barrier between blood and tissue, or through tissue. It may also be a complex function of more than one of these, and it may include a factor for plasma protein binding of the drug.