Hydroxyindole-*o*-methyltransferase IV: Inhibitory Activities of Some *N*-Acrylyltryptamines and *N*-Crotonyltryptamines

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Abstract □ Several *N*-acrylyltryptamines and *N*-crotonyltryptamines were synthesized to explore the mode of binding of the double bond in *N*-(1-cyclohexenecarbonyl)tryptamine to hydroxyindole-*o*-methyltransferase. Chlorine substitution at the β-position of the acrylyl or crotonyl group increased inhibition of the enzyme.

Keyphrases □ *N*-Acrylyltryptamines and *N*-crotonyltryptamines—synthesis □ *N*-(1-Cyclohexenecarbonyl)tryptamine double-bond binding—hydroxyindole-*o*-methyltransferase □ Chlorine substitution, *N*-acrylyltryptamines and *N*-crotonyltryptamines—hydroxyindole-*o*-methyltransferase inhibition □ IR spectrophotometry—structure □ NMR spectroscopy—structure

In previous works from this laboratory (1, 2), *N*-(1-cyclohexenecarbonyl)tryptamine (I) and *N*-benzoyltryptamine (II) were found to be, respectively, 3.4 and 2.3 times better inhibitors of hydroxyindole-*o*-methyltransferase (HIOMT) than *N*-cyclohexanecarbonyltryptamine (III). To explore the mode of binding of the carbon-carbon double bond in I, *N*-acrylyltryptamine (IV) and *N*-crotonyltryptamine (V) were synthesized. Since chlorine substitution on the *ortho* position of the phenyl group of II resulted in a sixfold increase in inhibitory activity (2), this study also includes Compounds VII–IX for investigating the possible enhancement of binding of the acrylyl (CH_2=CH—C(=O)—) and crotonyl (CH_3—CH=CH—C(=O)—) groups by a chlorine atom at the beta position.

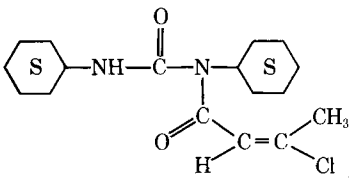
Results showed that IV and V were inhibitors of equal activity (Table I). The terminal CH_3 of the crotonyl moiety of V did not appear to contribute to the binding of this inhibitor to the enzyme. Substitution of the "methylene" hydrogen of the acrylyl group of IV by a chlorine atom slightly increased the activity (see VII). A greater increase in inhibition resulted when the chlorine atom was introduced to the "methine" carbon of the crotonyl moiety of V; VIII and IX were about twice as active as V.

The relative weakness in activities of those acrylyl and crotonyl derivatives compared to I could be attributed to the absence of several saturated methylene carbons which are capable of binding to the enzyme *via* hydrophobic interaction. The finding that *ortho*-chlorophenyl derivative (VI) is a much better inhibitor than the phenyl derivative (II), whereas the chlorocrotonyl derivatives (VII and VIII) are only slightly more active than the crotonyl derivative (V), can be visualized as a difference in the effect of a chlorine atom on the two series. A chlorine atom would be expected to exert stronger effect on the binding of an aromatic phenyl ring, as in the case of VI, than on an isolated double bond, as in VII or VIII. The observation that I was a better inhibitor than III would indicate that the C=C of the cyclohexenyl moiety of I provided additional binding to the enzyme. This type of unsaturated linkage is capable of complexing with the enzyme *via* a mixture of donor-acceptor, hydrogen-bonding, and even hydrophobic interactions (3).

PROCEDURES

N-Acyltryptamines IV, V, VII, VIII, and IX were prepared by the treatment of tryptamine with the appropriate acid chlorides obtained commercially or prepared from the corresponding acids. Attempts to prepare VIII from tryptamine and *trans*-3-chlorocrotonic acid in the presence of dicyclohexylcarbodiimide (DCC) were unsuccessful. Tryptamine and chlorocrotonic acid formed a salt which was insoluble in most organic solvents except aqueous tetrahydrofuran and aqueous acetonitrile. In the latter two cases, the only compound isolated from the reaction was the acylurea XI (m.p. 163–164°, MeOH $\nu_{max.}$ (KBr): 1700, 1645 (C=O); 1615 cm.$^{-1}$ (C=C).

Anal.—Calcd. for $C_{17}H_{27}ClN_2O_2$: C, 62.5; H, 8.33. Found: C, 62.4; H, 8.35.



XI

In the preparation of V, a second, higher melting crystalline compound was isolated. Dissimilarities in the IR spectra of the two products led to the initial assumption that one of them was disubstituted amide, *N*,*N*-dicrotonyltryptamine. However, elemental analyses indicated that they were isomeric, whereupon the melting points of both analytical samples were retaken. The compound, which originally had a m.p. of 83–84°, now melted over a range of 96–110°. When subjected to IR analysis, the spectrum of this substance changed to that of the higher melting compound in 2 hr. Further investigation was hampered by the unsuccessful attempts to isolate the lower melting product in later preparations.

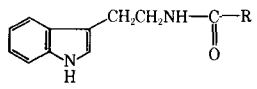
EXPERIMENTAL

Melting points are corrected and were taken on a melting-point apparatus.[1] IR spectra were obtained with a spectrophotometer.[2]

***N*-Acrylyltryptamine (IV)**—To a solution of 1.46 g. (10 mmoles) of tryptamine and 1.5 ml. (10.8 mmoles) of triethylamine in 75 ml. of chloroform was added, at 0°, 0.73 ml. (10.2 mmoles) of acrylyl chloride. The mixture was stirred at ambient temperature for 4 hr.; then 400 mg. (28%) of tryptamine hydrochloride was filtered off. The filtrate was extracted successively with 25-ml. portions of water, 2 *N* HCl, 2 *N* NaOH, and again water. The organic layer was dried (Na_2SO_4) and evaporated *in vacuo* to a yellow oil. Water was added to a solution of the oil in MeOH until turbidity occurred. The oily layer, which deposited upon standing, was separated by decantation and dissolved in 100 ml. of boiling water. After treating with char-

[1] Mel-Temp.
[2] Perkin-Elmer, model 237B.

Table I—Inhibition of HIOMT by



Compound	R	I_{50}[a] mM
I	1-Cyclohexenyl	0.25[b]
III	C_6H_{11}—	0.85[c]
II	C_6H_5—	0.37[c]
VI	*o*—ClC_6H_4—	0.055[b]
IV	$CH_2=CH$—	0.90
V	CH_3—$CH=CH$—	0.95
VII	Cl—$CH=CH$—(*trans*)	0.75
VIII	Cl, CH_3—$C=CH$—(*trans*)[d]	0.50
IX	Cl, CH_3—$C=CH$—(*cis*)	0.45

[a] Concentration of an inhibitor giving 50% inhibition of the enzyme. [b] Data from *Reference 2*. [c] Data from *Reference 1*. [d] *Trans* refers to the configuration in which the methyl and carbonyl groups are opposite one another.

coal (Norit) and filtering through diatomaceous earth (Celite), the solution was allowed to evaporate on bench top, yielding 650 mg. (31% after correction for recovery of tryptamine) of white, lustrous plates, m.p. 77–79°. Several recrystallizations from water gave an analytical sample, m.p. 79–80°; $\nu_{max.}$ (KBr): 1620 (C=O); 745 cm.$^{-1}$ (indole CH).

Anal.—Calcd. for $C_{13}H_{14}N_2O$: C, 72.9; H, 6.59; N, 13.1. Found: C, 72.6; H, 6.45; N, 13.0.

***N*-Crotonyltryptamine (V)**—In a similar manner as described in the preparation of IV, 10 mmoles of tryptamine was treated with crontoyl chloride in chloroform in the presence of triethylamine. After stirring at ambient temperature for 17 hr., the mixture was washed successively with water, 2 *N* HCl, 2 *N* NaOH, and water. The organic layer, after drying (Na_2SO_4), was evaporated *in vacuo*, leaving a yellow oil which did not crystallize. Distillation of this oil under reduced pressure yielded a pale-yellow, viscous oil which became semisolid upon setting, m.p. 76–78°. Extraction of the oily distillate with boiling water and subsequent evaporation of the extracts led to the formation of 660 mg. (30%) of small, lustrous plates, m.p. 78–80°. Several recrystallizations from water gave an analytical sample, m.p. 83–84°; $\nu_{max.}$ (KBr): 1620 (C=O); 745 cm.$^{-1}$ (indole CH).

Anal.—Calcd. for $C_{14}H_{16}N_2O$: C, 73.6; H, 7.06; N, 12.3. Found: C, 73.7; H, 7.10; N, 12.2.

Continuous extraction of this residual oil with boiling water yielded additional crops of crystals. The combined crops were recrystallized from water to give a higher melting V, m.p. 116–120°. An analytical sample was obtained upon further recrystallization of this product from water to a constant m.p. 129–130°; $\nu_{max.}$ (KBr): 1630 (C=O); 745 cm.$^{-1}$ (indole CH).

Anal.—Calcd. for $C_{14}H_{16}N_2O$: C, 73.6; H, 7.06; N, 12.3. Found: C, 73.9; H, 7.16; N, 12.3.

***N*-*trans*-(3-Chloroacrylyl)tryptamine (VII)**—A solution of 2.13 g. (20 mmoles) of *trans*-3-chloroacrylic acid and 3.0 ml. (23.5 mmoles) of benzenesulfonyl chloride in 15 ml. of xylene was heated at 90° for 30 min. and then distilled at 115–128°. This fraction of distillate, containing the acid chloride and xylene, was diluted with 15 ml. of chloroform and added at 0° to a solution of 3.2 g. (20 mmoles) of tryptamine and 4 ml. (29 mmoles) of triethylamine in 40 ml. of chloroform. The mixture was stirred at ambient temperature for 17 hr.; filtered to remove the tryptamine hydrochloride; and treated with water, 2 *N* HCl, 2 *N* NaOH, and water as described in the preparation of IV. The chloroform layer was dried (Na_2SO_4), treated with charcoal, filtered, and then evaporated *in vacuo*, leaving a pale-yellow semisolid. This product was dissolved in ether and treated with petroleum ether (b.p. 30–60°) to cloudiness. Upon chilling, 1.25 g. (25% after correction for recovery of tryptamine) of pale-yellow needles, m.p. 108–111°, was yielded. Subsequent recrystallization from ether–petroleum ether (b.p. 30–60°) gave an analytical sample as clumps of white needles, m.p. 109–110.5°; $\nu_{max.}$ (KBr): 1640 (C=O); 740 (indole CH).

Anal.—Calcd. for $C_{13}H_{13}ClN_2O$: C, 62.8; H, 5.27; Cl, 14.3; N, 11.3. Found: C, 62.7; H, 5.13; Cl, 14.0; N, 11.1.

***N*-*trans*-(3-Chlorocrotonyl)tryptamine (VIII)**—A mixture of 1.0 g. (8.3 mmoles) of *trans*-3-chlorocrotonic acid, 0.75 ml. (10.4 mmoles) of $SOCl_2$, and 6 ml. of $CHCl_3$ was refluxed for 45 min. After cooling, the solution was added to a chilled solution of 2.4 g. (15 mmoles) of tryptamine and 2.0 ml. (14.4 mmoles) of triethylamine in 25 ml. of $CHCl_3$. The mixture was stirred at ambient temperature for 4 hr., filtered to remove tryptamine hydrochloride, and concentrated to about 5 ml. Addition of 20 ml. acetone to this $CHCl_3$ solution precipitated a mixture of triethylamine hydrochloride and tryptamine hydrochloride which was removed by filtration. The acetone filtrate was evaporated *in vacuo* leaving an oil. A solution of this oil in chloroform was treated with water, 1 *N* HCl, 1 *N* NaOH, and water as described in the preparation of IV; dried (Na_2SO_4); treated with charcoal; and again evaporated *in vacuo*. The residual pale-yellow oil was extracted repeatedly with hot ether. When the combined ethereal extract was concentrated to about 20 ml., it turned cloudy and thick, shiny needles deposited upon standing on the bench top. Addition of petroleum ether (b.p. 30–60°) to this ethereal mixture caused the precipitation of more product; total first crop was 800 mg. (38%), m.p. 73–75.5°. Subsequent extractions of the original oil with ether afforded an additional crop of 390 mg. (18%). Thus, the total yield was 56%. Recrystallization of the first crop several times from ether–petroleum ether gave an analytical sample as shiny platelets, m.p. 77–78.5°; $\nu_{max.}$ (KBr): 1620 (C=O); 745 cm.$^{-1}$ (indole CH). NMR ($CDCl_3$): δ 2.18 (doublet, CH_3); 5.91 (doublet, olefinic H). Assignment of *trans* configuration was based on 3-chlorocrotonic acid (4).

Anal.—Calcd. for $C_{14}H_{15}ClN_2O$: C, 64.0; H, 5.75; N, 10.7. Found: C, 64.1; H, 5.86; N, 10.8.

***N*-*cis*-(3-Chlorocrotonyl)tryptamine (IX)**—The procedure was similar to that used for VII, except that the *cis*-3-chlorocrotonyl chloride was prepared by refluxing the corresponding acid with PCl_3 in xylene and distilled at 135–140°. The yellow oily product from the reaction was dried thoroughly *in vacuo* and then dissolved in 15 ml. of anhydrous ether. Concentration of the ethereal solution to about 5 ml. caused the deposition of a 44% yield (after correction for recovery of tryptamine hydrochloride) of yellow crystals, m.p. 98–101°. Addition of petroleum ether (b.p. 30–60°) to the filtrate of the first crop gave another 44% yield of less pure material, m.p. 89–93°. Recrystallization of the first crop several times from ether–petroleum ether afforded an analytical sample as transparent prisms, m.p. 102.5–103.5°; $\nu_{max.}$ (KBr): 1630 (C=O); 740 cm.$^{-1}$ (indole CH). NMR ($CDCl_3$): δ2.85 (doublet, CH_3); 5.83 (doublet, olefinic H). Assignment of *cis* configuration was based on 3-chloroisocrotonic acid (4).

Anal.—Calcd. for $C_{14}H_{15}ClN_2O$: C, 64.0; H, 5.75; N, 10.7. Found: C, 63.9; H, 5.67; N, 10.6.

Assay—Hydroxyindole-*o*-methyltransferase was isolated from beef pineal gland and purified according to the method of Axelrod and Weissbach (5).

The stock solutions of all the inhibitors were prepared in 50% aqueous propylene glycol. Incubation was carried out with *N*-acetylserotonin and *S*-adenosyl-L-methionine-methyl-^{14}C according to the previously described procedure (6).

REFERENCES

(1) B. T. Ho, W. M. McIsaac, L. W. Tansey, and P. M. Kralik, *J. Pharm. Sci.*, **57**, 1998(1968).

(2) B. T. Ho, W. M. McIsaac, and L. W. Tansey, *ibid.*, **58**, 563 (1969).

(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967, p. 44.

(4) "High Resolution NMR Spectra Catalog," vol. 1, Varian, Palo Alto, Calif., 1962, Spectra Nos. 53 and 54.

(5) J. Axelrod and H. Weissbach, *J. Biol. Chem.*, **236**, 211(1961).

(6) B. T. Ho, W. M. McIsaac, and L. W. Tansey, *J. Pharm. Sci.*, **58**, 130(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 9, 1969, from the *Texas Research Institute of Mental Sciences, Houston, TX 77025*

Accepted for publication December 11, 1969.

This work was supported by Grants MH-11168 and MH-12959, U. S. Public Health Service, and the Britton Fund.

The authors thank Miss Patricia M. Kralik and Mr. Edward Fritchie for the enzyme assays and Miss Geraldine Hagger for technical assistance.