on cytochrome P-450 and aminopyrine *N*-demethylase activity in rat hepatic microsomes. *Biochem Pharmacol* **33**: 3277–3281, 1984.
13. Murray M and Farrell GC, Effect of primaquine on hepatic microsomal haemoproteins and drug oxidation. *Toxicology* **42**: 205–217, 1986.
14. Riviere JH and Back DJ, Effect of mefloquine on hepatic metabolism in the rat: comparative study with primaquine. *Biochem Pharmacol* **34**: 567–571, 1985.
15. Riviere JH and Back DJ, Inhibition of ethinyloestradiol and tolbutamide metabolism by quinoline derivatives *in vitro*. *Chem Biol Interact* **59**: 301–308, 1986.
16. Na Bangchang K, Karbwang J and Back DJ, Mefloquine metabolism by human liver microsomes: effect of other antimalarial drugs. *Biochem Pharmacol* **43**: 1957–1961, 1992.
17. Purba HS, Maggs JL, Orme ML'E, Back DJ and Park BK. The metabolism of 17α-ethinyloestradiol by human liver microsomes: formation of catechol and chemically reactive metabolites. *Br J Clin Pharmacol* **23**: 447–453, 1987.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
19. Omura T and Sato R, The carbon monoxide binding pigment of liver microsomes. *J Biol Chem* **239**: 2370–2378, 1964.
20. Webb JL, Determination of mechanisms and constants of inhibition. In: *Enzyme and Metabolic Inhibitors*, Vol. 1, pp. 141–191. Academic Press, New York, 1963.
21. Dixon M, The determination of enzyme inhibitor constants. *Biol J* **55**: 170–171, 1953.
22. White NJ, Clinical pharmacokinetics of antimalarial drugs. *Clin Pharmacokinet* **10**: 187–215, 1985.
23. Karbwang J and White NJ, Clinical pharmacokinetics of mefloquine. *Clin Pharmacokinet* **19**: 264–279, 1990.
24. Resetar A, Minick D and Spector T, Glucuronidation of 3'-azido-3'-deoxythymidine catalysed by human liver UDP-glucuronosyltransferase. Significance of nucleoside hydrophobicity and inhibition by xenobiotics. *Biochem Pharmacol* **42**: 559–568, 1991.
25. Sonino N, The use of ketoconazole as an inhibitor of steroid production. *N Engl J Med* **317**: 812–818, 1987.

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Inhibitory potency of some isatin analogues on human monoamine oxidase A and B

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Abstract—Isatin is an endogenous compound which acts as a selective inhibitor of monoamine oxidase (MAO) B. In this study a range of isatin analogues were tested for their *in vitro* inhibition of human MAO A and B. Most of the analogues were less potent than isatin. Hydroxylation of the aromatic ring changed the inhibitory potency in favour of MAO A, with 5-hydroxyisatin being a potent and selective MAO A inhibitor (IC_{50} 8 μM). Isatinic acid, which is formed reversibly from isatin at alkaline pH, showed no inhibition.

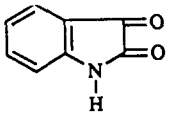
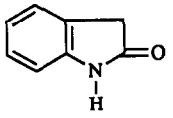
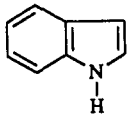
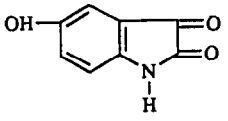
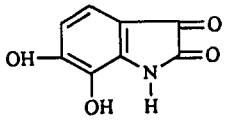
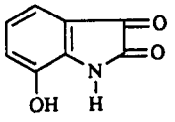
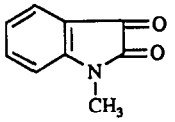
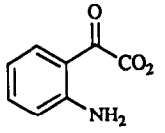
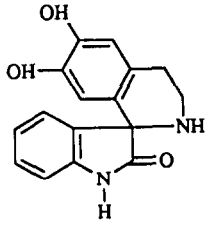
Isatin has long been known as a pharmacologically active agent which exerts a number of *in vitro* and *in vivo* effects (for review, see Ref. 1). In particular, it is anxiogenic at low doses in rodents [1, 2]. It has recently emerged as a major constituent of tribulin, a naturally occurring, low molecular mass inhibitor of monoamine oxidase (MAO) and benzodiazepine binding [3–5], the output of which is increased in various states of stress or anxiety (e.g. Refs 6, 7). However, isatin does not account for all the activity ascribed to tribulin [8]. Isatin is a more potent inhibitor of MAO B than MAO A [5], whereas inhibition by tribulin is equipotent [4]. Isatin is also a relatively weak inhibitor of benzodiazepine receptor binding compared with tribulin [9].

Such discrepancies between the inhibitory properties of isatin and tribulin point to the presence of as yet unidentified components of tribulin, which can selectively inhibit MAO A and also inhibit benzodiazepine binding more potently. In the present study, we have examined the MAO inhibitory effect of hydroxylated and other analogues of isatin, including a *spiro*-tetrahydroisoquinoline condensation product of dopamine and isatin (dopamine–isatin), *N*-methylisatin, indole and oxindole, to try to understand how chemical modification of isatin affects inhibitory activity. We have also investigated the effect of isatinic acid, formed reversibly from isatin at alkaline pH.

Materials and Methods

The inhibitory effect of isatin and its analogues was tested on human placental MAO A and human platelet MAO B, using 170 μM [^{14}C]5-hydroxytryptamine (sp. act. 2.5 μCi/μmol) and 5 μM [^{14}C]phenylethylamine (sp. act. 12.5 μCi/μmol), respectively, as described previously [10]. These concentrations, which are close to the K_m value, allow competitive inhibition to be detected. IC_{50} values for the compounds tested were calculated from their inhibition curves, using a range of concentrations from 10^{-8} to 10^{-4} M for each compound. The results represent means of at least six independent experiments, with a standard error of less than 5%. Radiolabelled 5-hydroxytryptamine and

Table 1. Inhibition of human MAO A and B by isatin and related compounds

Structure	Name	IC_{50} (μM) MAO A	IC_{50} (μM) MAO B
	Isatin	56 ± 0.2	7.9 ± 0.4
	Oxindole	⩾100	73 ± 13
	Indole	100	100
	5-Hydroxyisatin	8.4 ± 1.4	>100
	6-Hydroxyisatin	100	>100
	7-Hydroxyisatin	100	>100
	*N*-Methylisatin	95 ± 3	14 ± 3
	Isatinic acid	⩾100	⩾100
	Dopamine–isatin	⩾100	⩾100

phenylethylamine were obtained from the Radiochemical Centre (Amersham, U.K.). Other chemicals were purchased from the Sigma Chemical Co. (Poole, U.K.) and the Aldrich Chemical Co. (Gillingham, U.K.). Dopamine–isatin was prepared by the standard Pictet–Spengler reaction [11]. Other isatin derivatives were prepared by standard methods [12–15], modified where necessary for preparation of hydroxy- and N-substituted isatins. Their structures were confirmed by mass spectral analysis. Isatinic acid was prepared by incubating isatin for 20 min at pH 8.5 in 100 mM phosphate buffer; the pH was readjusted to pH 7.4 immediately before assay.

Results and Discussion

The results are given in Table 1. The inhibitory potency of the compounds, tested against MAO B, increased in the following order: isatinic acid = dopamine–isatin < 6 hydroxyisatin < 7-hydroxyisatin < 5-hydroxyisatin < indole < oxindole < *N*-methylisatin < isatin. When these compounds were incubated with MAO A, the following rank order of inhibitory activity was obtained: isatinic acid < oxindole < dopamine–isatin < indole = 7-hydroxyisatin = 6-hydroxyisatin < *N*-methylisatin < isatin < 5-hydroxyisatin. Hydroxylation of the aromatic ring changed the inhibitory potency of all the isatin analogues in favour of MAO A. 5-, 6- and 7-Hydroxyisatin all showed selective MAO A inhibition, although only 5-hydroxyisatin acted as a more potent inhibitor of MAO A than isatin itself. Thus, hydroxylation of the isatin molecule at position 5 created a selective and potent MAO A inhibitor. It is of interest that, in this context, hydroxylation of tryptamine in the 5 position results in a marked shift in substrate specificity from MAO B to MAO A [16].

The data in Table 1 also indicate that insertion of oxo group(s) into the indole ring increases inhibitory potency towards MAO B (indole < oxindole < isatin), but not towards MAO A where indole was more potent than oxindole. The 3-oxo group appears to have the greatest inhibitory potency on both forms of the enzyme. This is not surprising as the 3-oxo group is highly reactive whilst, in general, the 2-oxo (amide) group is unreactive. It is probable that the 3-oxo group forms a Schiff's base with a free amino group in the MAO molecule.

It is of particular interest that isatinic acid showed little inhibitory activity towards either form of the enzyme even at 10^{-4} M. It is not clear in what form isatin exists *in vivo*. We have found that an isatin solution left overnight in phosphate buffer, pH 7.4, loses both colour and MAO inhibitory potency due to the formation of isatinic acid which can be reversed by acidification (unpublished observations). If isatin exists predominantly as isatinic acid *in vivo*, then the possible mechanism of its anxiogenic and other properties [2] may require re-evaluation.

Of the compounds tested here, only 5-hydroxyisatin could be a candidate for the additional MAO A inhibitory component of tribulin. In preliminary experiments, we have shown, using reverse phase HPLC, that partially purified urinary tribulin can be resolved into several peaks, one of which is a selective inhibitor of MAO A with chromatographic properties similar to those of 5-hydroxyisatin; however, mass spectrometric analysis of this sample failed to confirm its presence (unpublished). The identity of endogenous selective MAO A inhibitor(s) thus remains unknown.

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REFERENCES

1. Glover V, Bhattacharya SK and Sandler M, Isatin—a new biological factor. *Indian J Exp Biol* **29**: 1–5, 1991.
2. Bhattacharya SK, Mitra SK and Acharya SB, Anxiogenic activity of isatin, a putative biological factor, in rodents. *J Psychopharmacol* **5**: 202–206, 1991.
3. Sandler M, The emergence of tribulin. *Trends Pharmacol Sci* **3**: 471–472, 1982.
4. Elsworth JD, Dewar D, Glover V, Goodwin BL, Clow A and Sandler M, Purification and characterization of tribulin, an endogenous inhibitor of monoamine oxidase and of benzodiazepine receptor binding. *J Neural Transm* **67**: 45–56, 1986.
5. Glover V, Halket J, Watkins PJ, Clow A, Goodwin BL and Sandler M, Isatin: identity with the purified endogenous monoamine oxidase inhibitor tribulin. *J Neurochem* **51**: 656–659, 1988.
6. Glover V, Bhattacharya SK, Sandler M and File SE, Benzodiazepines reduce stress-augmented increase in rat urine monoamine oxidase inhibitor. *Nature* **292**: 347–349, 1981.
7. Clow A, Glover V, Sandler M and Tiller J, Increased urinary tribulin output in generalised anxiety disorder. *Psychopharmacology* **95**: 378–380, 1988.
8. Watkins P, Clow A, Glover V, Halket J, Przyborowska A and Sandler M, Isatin, regional distribution in rat brain and tissues. *Neurochem Int* **17**: 321–323, 1990.
9. Armando I, Glover V and Sandler M, Distribution of endogenous benzodiazepine receptor ligand–monoamine oxidase inhibitory activity (tribulin) in tissues. *Life Sci* **38**: 2063–2067, 1986.
10. Glover V, Reveley M and Sandler M, A monoamine oxidase inhibitor in human urine. *Biochem Pharmacol* **29**: 467–470, 1980.
11. Whaley WM and Govindachari TR, The Pictet–Spengler synthesis of tetrahydroisoquinolines and related compounds. In: *Organic Reactions* (Eds. Adams R, Adkins H, Blatt AM, Cope AC, McGrew FC, Niemann C and Snyder HR), Vol. 6, pp. 151–190. Wiley, New York, 1951.
12. Marvel CS and Hiers GS, Isatin. *Org Syn Coll* **I**: 321–324, 1941.
13. Bauer DJ and Sadler PW, Structure–activity relations of the antiviral chemotherapeutic activity of isatin β-thiosemicarbazone. *Br J Pharmacol* **10**: 1–10, 1960.
14. Sadler PW, Separation of isomeric isatins. *J Org Chem* **21**: 169–170, 1956.
15. Gripenberg J, Honkanen E and Patoharju O, Fungus pigments. V. Degradation of annabaris. *Acta Chem Scand* **11**: 1485–1492, 1957.
16. Kuhn DM, Murphy DL and Youdim MBH, Physiological and clinical aspects of monoamine oxidase. In: *Structure and Functions of Amine Oxidases* (Ed. Mondovi B), pp. 231–248. CRC Press, Florida, 1985.