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Note

Isotopic fractionation of N-([^{3}H]methyl)-chlorpromazine and N-([^{3}H]methyl)-7-hydroxychlorpromazine by reversed-phase high-performance liquid chromatography

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During a metabolic study of the phenothiazine antipsychotic chlorpromazine and its metabolite 7-hydroxychlorpromazine (Fig. 1), it was noted that the N-([^{3}H]methyl)-analogoues of the two compounds were resolved from the cold standards when they were analyzed by high-performance liquid chromatography (HPLC). Isotopic fractionation by HPLC has been reported for mianserin and its derivatives[1,2]. Separation of the isotopic species were achieved with a normal-phase HPLC system. In the present technical report, a reversed-phase system was used in which it was found that the separation of the isotopic species was pH dependent.

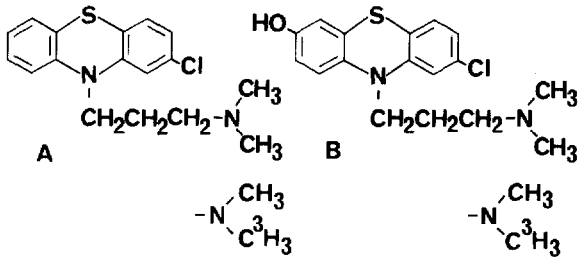

Fig. 1. Structures of chlorpromazine (A), 7-hydroxychlorpromazine (B) and their N-([^{3}H]methyl)-analogues.

EXPERIMENTAL

N-([^{3}H]Methyl)-chlorpromazine (specific activity 44 Ci/mmol) was prepared by N-methylation of chlorpromazine with tritiated methyliodide (specific activity ap-

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proximately 87 Ci/mmol), and subsequent demethylation of the tritiated quarternary ammounium species with diazabicyclo[2.2.2.]octane. N-([^{3}H]methyl)-7-hydroxychlorpromazine (specific activity 44 Ci/mmol) was synthesized from N-([^{3}H]methyl)-chlorpromazine by an enzymatic method[3]. ^{3}H-Benzene ring labelled chlorpromazine (30 Ci/mmol) was purchased from New England Nuclear Canada (Lachine, Quebec, Canada). Non-tritiated reference standards of chlorpromazine and 7-hydroxychlorpromazine were obtained from Rhone-Poulenc (Montreal, Quebec, Canada) and Dr. A. A. Manian of the National Institute of Mental Health (Rockville, MD, U.S.A.), respectively.

The HPLC system consisted of a liquid chromatographic pump (Model 45, Waters Assoc., Milford, MA, U.S.A.), a valve-loop injector fitted with a 1-ml loop (Model 7120, Rheodyne Technical Marketing Assoc., Ottawa, Ontario, Canada), a 250 × 4.6 mm I.D. column packed with 5-μm cyanobonded column packing (Spherisorb CN, Altex, Beckman Instruments, Toronto, Ontario, Canada), and a variable-wavelength UV detector (Model 480, Waters Assoc.). Resolution of the isotopic species was studied with a 10% 0.05 *M* sodium acetate buffer in methanol system, in which the pH of the buffer solution was adjusted to 5, 6 or 7. The system was operated at ambient temperature and at a flow-rate of 1.5 ml/min. Radioactivity was determined in a LKB Rackbeta liquid scintillation counter equipped with an automatic quench compensation (Model 1215, Fisher Scientific, Edmonton, Alberta, Canada).

RESULTS AND DISCUSSION

Specific activity of the N-([^{3}H]methyl)-chlorpromazine product prepared as described was calculated to be 44 Ci/mmol. This indicated that the product was a mixture of N-([^{3}H]methyl)-chlorpromazine and some residual unlabelled chlorpromazine. If every chlorpromazine molecule was incorporated with a tritiated methyl group, the specific activity of the labelled product would be equal to that of the tritiated methyl iodide which was approximately 87 Ci/mol. Production of tritiated chlorpromazine with this specific activity is unlikely with the described synthetic route. Assuming there was no isotopic effect in the N-demethylation of the tritiated quaternary ammonium chlorpromazine species, the ratio of unlabelled to labelled chlorpromazine in the final product would be 1:2. Thus the theoretical specific activity of the labelled chlorpromazine product would be 2/3 × 87 = 58 Ci/mmol. The observed specific activity was 44 Ci/mmol.

HPLC chromatograms of this mixture obtained with three different HPLC solvent systems, are shown in Fig. 2. In the solvent system made with pH 5.0 buffer, the mixture gave a single HPLC peak (Fig. 2C) and a retention volume of 17.61 ml. This was the same retention volume as the unlabelled chlorpromazine standard. In solvent systems containing buffer at higher pH values, however, the mixture was resolved into its two components. Peak I in the HPLC traces occurred at the same retention volume as unlabelled chlorpromazine (13.26 ml with buffer at pH 7.0, Fig. 2A; 17.07 ml with buffer at pH 6.0, Fig. 2B). A fraction of the eluate containing the compound giving rise to peak I (Fig. 2A, retention volume 13.26 ml) contained only 3% of the radioactivity in the fraction corresponding to peak II. The small amount of radioactivity in this fraction was probably due to contamination from the second component because the HPLC system did not separate the components of the mixture

TABLE I

CHROMATOGRAPHIC PARAMETERS OF CHLORPROMAZINE (CPZ), 7-HYDROXYCHLORPROMAZINE (7-OHCPZ), AND THEIR TRITIATED ANALOGUES

Solvent	*CPZ retention volume (V_1) (ml)*	*N-([^{3}H]methyl)-CPZ retention volume (V_2) (ml)*	*[^{3}H-]Benzene ring labelled-CPZ retention volume (V_3) (ml)*	$\alpha \frac{V_2 - V_0^*}{V_1 - V_0}$	*7-OHCPZ retention volume (V_1) (ml)*	*N-([^{3}H]methyl)-7-OHCPZ retention volume (V_2) (ml)*	$\alpha \frac{V_2 - V_0}{V_1 - V_0}$
10% 0.05 *M* sodium acetate buffer (pH 7.0) in methanol	13.26	15.00	13.26	1.17	11.43	13.02	1.18
10% 0.05 *M* sodium acetate buffer (pH 6.0) in methanol	17.07	18.66	17.07	1.11	13.89	14.94	1.10
10% 0.05 *M* sodium acetate buffer (pH 5.0) in methanol	17.61	17.61	17.61	1	15.03	15.69	1.05

* V_0 = Void volume.

completely. Thus peak I in the chromatograms (Fig. 2A and B) corresponds to unlabelled chlorpromazine and peak II corresponds to N-([^{3}H]methyl)-chlorpromazine. The specific activity of the latter was 82 Ci/mmol which was close to that of tritiated methyl iodide (87 Ci/mmol).

Isotopic fractionation also occurred between unlabelled 7-hydroxychlorpromazine and N-([^{3}H]methyl)-7-hydroxychlorpromazine. As shown in Table I, the selectivity coefficient (α) increased when the pH of the sodium acetate buffer solution was changed from 5 to 7. Using pH 5 buffer, a slight separation (α = 1.05) was observed for the isotopic species of 7-hydroxychlorpromazine. The retention volumes of both isotopic species were smaller at higher pH (Table I). The effect of pH on isotopic fractionation has previously been observed for several ^{14}C-labelled amino acids and tritiated aminopurine using open column ion-exchange chromatography[4]. In the case of amino acids, separations were more apparent when the ^{14}C atom was adjacent to an ionizable group. It was suggested that the separation was due to a change in the pK_a values as a result of the isotope changes. De Ridder and Van Hal[2] suggested that a shielding effect on the nitrogen atoms by deuterium might account for the separation of a mianserin derivative (Org GC 94) from its tetradeuterated analogue on a normal-phase HPLC system. These authors implied further that deuterium atoms in the nearby vicinity of the nitrogen atoms might alter the pK_a of the mianserin derivatives because the separation was affected by the concentration of ammonia in the mobile phase. The present investigation was carried out in a reversed-phase system, and the pH effect on the isotopic fractionation also suggests an alteration of pK_a values as a result of the introduction of tritium atoms on the N-methyl group of chlorpromazine and 7-hydroxychlorpromazine. If ionic species were eluted later than non-ionic species, as was suggested by the larger retention volume at lower pH values, an effect of the tritium labelling might have been an increase in the pK_a values, since the labelled species were eluted later than the cold standards. Under sufficiently acidic conditions, such as in the solvent system containing 0.05 *M* pH 5

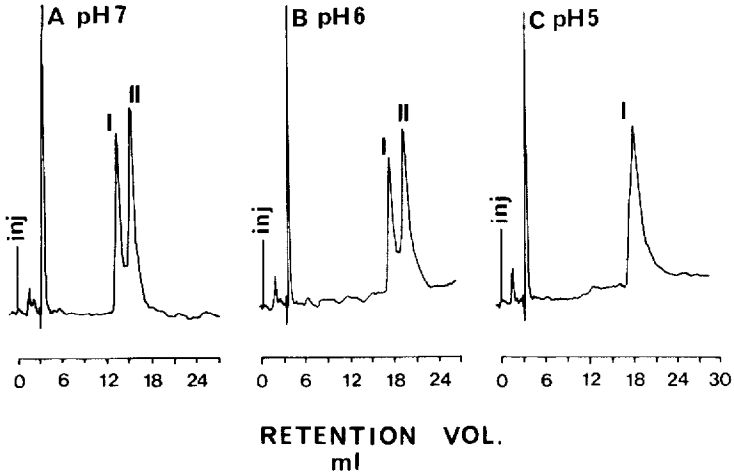

Fig. 2. HPLC–UV chromatograms of N-([^{3}H]methyl)-chlorpromazine using three different mobile phases. (A) 10% 0.05 *M* pH 7 sodium acetate buffer in methanol; (B) 10% 0.05 *M* pH 6 sodium acetate buffer in methanol; (C) 10% 0.05 *M* pH 5 sodium acetate buffer in methanol.

sodium acetate buffer, the non-tritiated species were protonated to the same extent as the tritiated analogs such that they were not resolved by the chromatography (Fig. 2C). On the other hand, chlorpromazine with tritium atoms on the benzene ring (specific activity approximately 30 Ci/mmol) was chromatographed as a single peak with retention volumes identical to cold chlorpromazine standard under the three different pH conditions as described above (Table I). This further supports the view that alteration in the pK_a values would probably occur only when tritium atoms are in close vicinity to the nitrogen atom.

The described HPLC method provides a means for isotopic enrichment of the type of tritiated organic compounds discussed here. The data presented here suggest that extra care must be taken in interpreting radiochemical purity as determined by HPLC. From the observation that different chromatographic behaviors were exhibited between the N-([^{3}H]methyl)-analogues of chlorpromazine and 7-hydroxychlorpromazine as compared to the cold standards when they were analyzed at pH 7, it is tempting to speculate that these tritiated analogues may show different pharmacological properties at physiological condition. Thus selection of sites for the tritium labelling must be carefully considered especially in studies such as receptor binding, metabolism and radioimmuno-assays.

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REFERENCES

1 C. N. Filer, R. Fazio and D. G. Ahern, *J. Org. Chem.*, 46 (1981) 3344.
2 J. J. De Ridder and J. M. Van Hal, *J. Chromatogr.*, 121 (1976) 96.
3 P. K. F. Yeung, G. McKay, I. A. Ramshaw, J. W. Hubbard and K. K. Midha, *J. Pharmacol. Exp. Ther.*, submitted for publication.
4 P. D. Klein, *Adv. Chromatogr.*, 3 (1966) 23.