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ACID-CATALYSED DEUTERIUM EXCHANGE OF AROMATIC PROTONS. PART III[1]: ACCELERATED EXCHANGE BY MICROWAVE IRRADIATION.

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SUMMARY

Conventional acid-catalysed $^{2}H/^{1}H$ exchange in aromatic rings requires long reaction times, high temperatures and pressure. This paper reports that accelerated deuterium exchange can be achieved in a microwave oven. Experiments were carried out on benzodiazepines, tricyclic antidepressants and phenothiazines. The reaction time was decreased from days to minutes, the preparatory work was simpler than with conventional heating and the labelled products were cleaner.

Key Words: Deuterium exchange. Microwave - induced reaction, Aromatic proton exchange. Drug deuterium labelling.

INTRODUCTION

In recent years, several authors have proposed acid catalysed $^{2}H/^{1}H$ exchange of aromatic protons on simple aromatic rings (2-4). Using a similar method, benzodiazepines and their precursors were labelled and the deuterium labelling of other drugs was reviewed (5-6).

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The authors (2-6) recommended high temperature (max 300°C), elevated pressure and a long reaction time (up to 4 days) for the $^{2}H/^{1}H$ exchange.

The use of a microwave for general chemical reactions has recently been reviewed by Abramowitch (7). Methods have also been reported for the synthesis of ^{11}C labelled compounds utilizing microwave (8-9). The authors reported very short reaction times and clean reaction products. The use of "coaxial resonance microwave cavity" further accelerated the reaction in a small sample size (8-10). The reaction kinetics using microwave irradiation - in a specially modified oven - and conventional heating were compared by Raner et al. (11). The authors indicated that no special "microwave effect" exists in the case of accelerated reactions. The rate of the reactions was the same in both cases.

The application of microwaves in chemical syntheses (7-10) led to the implementation of this simple, practical method for the acid-catalysed deuterium exchange of selected drugs. The successfully labelled drugs are under investigation for use as internal standards for GC/MS/SIM quantitative analyses in this laboratory.

RESULTS and DISCUSSION

In one study, several tricyclic antidepressant drugs were treated with ^{2}HCl and conventional heating; the reaction times were 8 - 16 hours at 160°C (12). However, no literature was available regarding the deuterium labelling of phenothiazines. Therefore, to obtain some information on this class of drugs and to be able to compare with the microwaves method, phenothiazine and perphenazine were also labelled by the conventional heating method. The labelling time for phenothiazine and perphenazine at 150°C were 6 and 4 hours respectively.

The initial experiments were carried out on 1.0 mg each of alprazolam, triazolam and substituted benzophenones (precursors for benzodiazepines) in a mixture of $AcO^{2}H:^{2}H_{2}SO_{4}:^{2}H_{2}O$ (2:1:1). The acid mixture and reaction time for the labelled products were established in previous experiments (5-6). In the microwave oven, significant deuterium exchange was measured in minutes, as shown in Table 1.

Experiments with tricyclic antidepressants and phenothiazines in concentrated ^{2}HCl solution were more surprising (Table 2). The $^{2}H/^{1}H$ exchange was more complete as compared with the benzodiazepine

Table 1 - Deuterium Labelling of Benzodiazepines and their Precursors

Drug Name	No of $^2H/^1H$ exchange[a]	No of $^2H/^1H$ exchange[b]
Alprazolam	4	2
Triazolam	4	6
2-NH_2-5-Cl-benzophenone	2	2
2-NH_2-5,5'-2Cl-benzophenone	3	3

[a]5 minutes irradiation, [b]4 days/200°C conventional heating

Table 2 - Deuterium Labelling of Tricyclic Antidepressants and Phenothiazines in Microwave Oven

Drug Name	No of $^2H/^1H$ exchange[a]	Drug Name	No of $^2H/^1H$ exchange[b]
Iminodibenzyl	4	Phenothiazine	4
Iminostilbene	4	Chlorpromazine	4
Amitriptyline	1	Promazine	4
Clomipramine	3	Fluphenazine	1
Cyclobenzaprine	3	Trifluperazine	1
Desipramine	3	Methotrimeprazine	4
Imipramine	3	Thioridazine	1
Nortriptyline	1	Thiopropazate	4
Protriptyline	1	Perphenazine	4
Trimipramine	4		

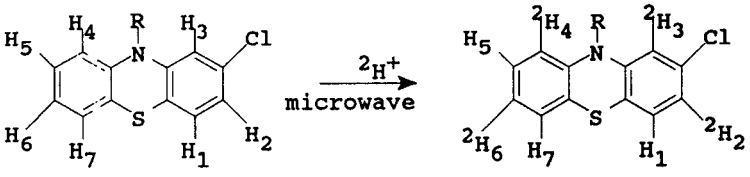
[a]5 minutes irradiation, [b]1 minute irradiation

class and the mass spectra isotope envelope of the product was isotopically cleaner. Optimization of the exchange parameters with different acids and acid mixtures is under investigation.

The labelling of perphenazine was particularly important for this laboratory. Since postmortem blood concentrations of perphenazine are in the ng/mL (0.3-25) range, the MS/SIM method is recommended for quantitative analysis and the use of a deuterated homolog as internal standard is required. Therefore, 100 mg of labelled perphenazine was synthesised. Perphenazine deuterium labelling was carried out in a 23 mL Parr® teflon bomb. The Parr operating instruction indicated that if 20 mL of concentrated HCl (36%) acid is heated to 220°C in a closed 45 mL bomb, the pressure

will reach 1430 psi, which is well above the maximum limit for this vessel. To avoid overheating and excessive pressure build-up during the reaction, a shorter exposure time (1.5 minutes repeated once) was selected and the amount of acid was reduced.

After 30 seconds the exchange was 90 %. With an additional irradiation of 5 x 30 second, the $^{2}H/^{1}H$ exchange was 99.5%. The MS electron ionization spectrum showed 4 proton exchanges in the phenothiazine ring (Fig. 2. B-D). The ^{1}H NMR in $C^{2}HCl_3$ solution has shown three unexchanged protons, as two duplets and one singlet, which indicated a loss of 4 protons from the original seven (Fig. 2. A-C). The location of the exchange in the phenothiazine ring (Fig. 1) was determined by comparative interpretation of the original ring system and decoupling test.



R = propyl-1-piperazineethanol

Fig. 1 - Location of the Deuterium Exchange in Perphenazine

CONCLUSION

The use of microwave irradiation to accelerate the $^{2}H/^{1}H$ exchange in sealed vessels is valuable. However, as with every new technique, the optimum for the parameters that affect the exchange process have to be investigated. The method presented here has the drawback of the possibility of random exchange as in conventional thermal heating and the lack of control of temperature and pressure in the reaction vessel. Despite these difficulties, speedy results make this method preferable to conventional ones. The proper catalysts and the optimal parameters are under investigation for benzodiazepines, tricyclics, phenothiazines and other drugs, which have proton exchangeable aromatic structures.

EXPERIMENTAL

USP quality unlabelled drugs were obtained from various drug manufacturing companies and the ^{2}H labelled chemicals were purchased from MSD Isotopes (Dorval, Quebec, Canada). The GC/MS analyses were carried out on a Hewlett Packard (HP) ENGINE PARTICLE BEAM system

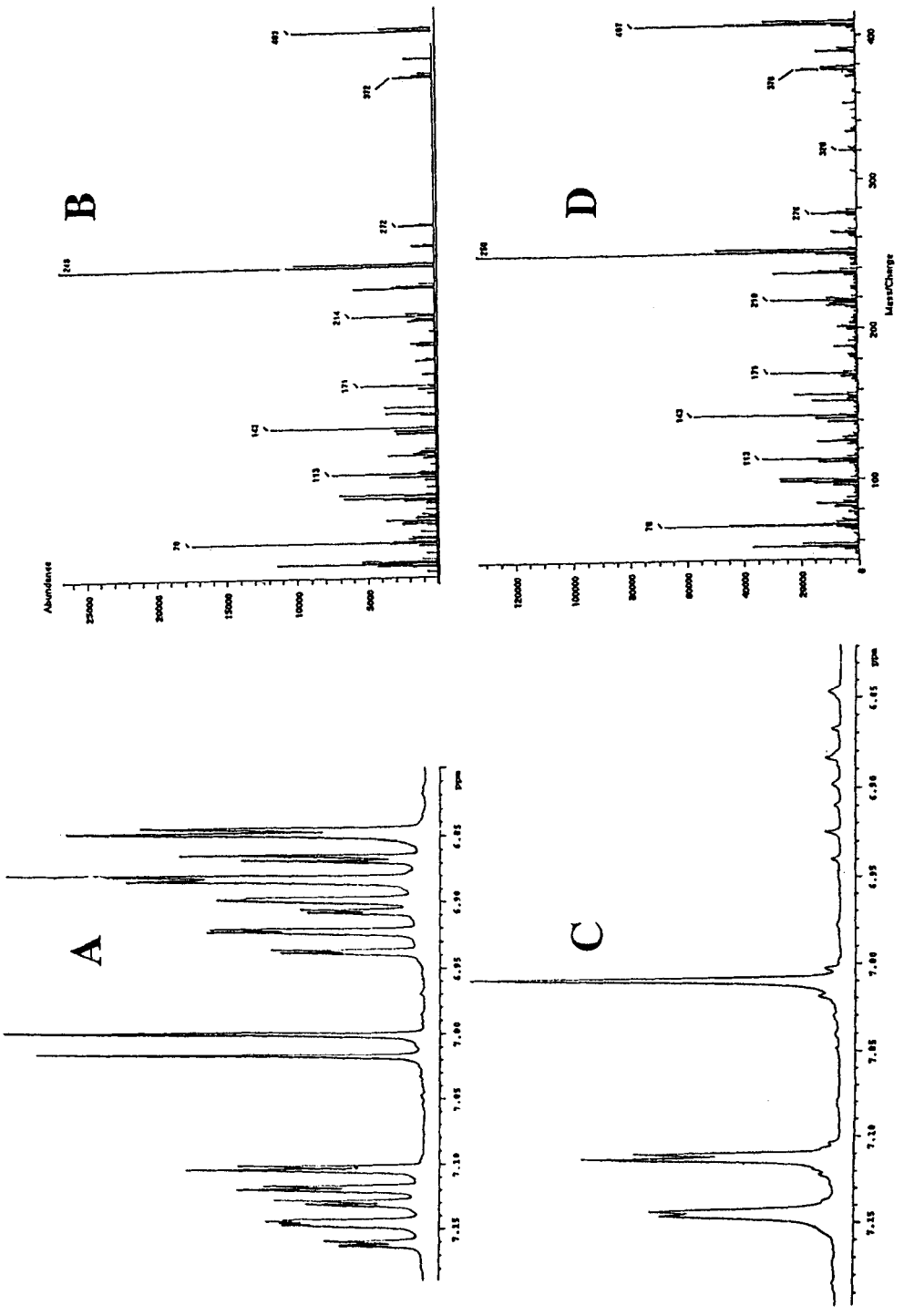


Figure 2. - A: ^1H NMR of Perphenazine, **B:** MS of Perphenazine, **C:** ^1H NMR of Perphenazine-d_4, **D:** MS of Perphenazine-d_4

in EI and CI^- mode. Chemical ionization was achieved using methane gas. The gas chromatograph was fitted with a DB-1 fused silica capillary column (30m x 0.25mm i.d., 0.1μm film thickness, J&W Scientific Inc.). The HPLC analyses were carried out on the same system using a Diode Array Detector (DAD) in combination with the mass spectrometer (LC/DAD/MS). The column was a HP Hypersil C18 analytical column (5μm particle size, 100mm x 2.1mm ID) and the mobile phase was 25 % aqueous 0.1 M ammonium acetate and 75 % of a mixture of MeCN:MeOH (10:90). The flow rate was 0.3 mL/min. The UV/DAD detector measured in the 220-360 nm interval, with 4 nm steps, and 0.5 mAU threshold. The ^1H NMR spectra were obtained in C^2HCl_3 on a BRUKER 500 MHz instrument. The test reactions were carried out in 5 ml "Reacti-Vials®", sealed with Teflon-Rubber Laminated Disc (Pierce. Rockford, IL. USA.) in a GM-MICROWAVE OVEN (Model JEM31LWC), with 800 W energy, delivered to the oven.
The 100 mg perphenazine $^2H/^1H$ exchange was carried out in a Parr® teflon bomb (Model 4781).

TYPICAL TESTING REACTIONS: From 10 mg/10 ml of drug solution in methanol, 1 ml was measured into a 5 ml Reacti-Vial® and evaporated under a stream of nitrogen. The residue was reconstituted with 0.1 mL of the appropriate acid mixture. The vials were securely closed and placed in the center of the microwave oven. At the high energy setting, the samples were irradiated in 30 second bursts (in 3 minutes interval) to the total times listed in Tables 1 and 2. A 10 μL sample was added to 500 μL deionized water and made alkaline with 50 μL of 5N NaOH. The mixture was extracted 3 times with 500 μL of methylene chloride. The combined organic layer was washed with 500 μL of deionized water, dried on Na_2SO_4 and evaporated. The residue was reconstituted in 1.0 ml toluene and 2 μL was injected onto the GC/MS instrument. For LC/DAD/MS analysis, the residue was reconstituted in 1 mL of mobile phase and 25 μL was injected.

PERPHENAZINE $^2H/^1H$ EXCHANGE IN 100 MG SCALE: One hundred mg of perphenazine was dissolved in 5 mL of conc. ^2HCl in the 23 mL PARR teflon bomb and placed in the center of the microwave oven. The high energy setting was used twice for 1.5 minutes each time. After cooling the reaction mixture (3-5 min), it was transferred into an Erlenmeyer flask and kept in an ice bath. The bomb was rinsed with 10 mL of deionized water which was then combined with the reaction mixture in the Erlenmeyer flask. After the addition of 10 mL 5N NaOH it was extracted with 10 mL of methylene chloride. The extraction was repeated three times with 5 mL of methylene

chloride. The combined organic layer was washed with 5 mL of deionized water, dried on Na_2SO4 and evaporated in vacuo in a rotary evaporator. The oily product was purified on Merck Kieselgel 60F plate (20 x 20cm, thickness 2.0mm). The developing solution was 175 mL of toluene, 80 mL of chloroform, 40 mL of ethanol and 2 mL of conc. ammonium hydroxide. The appropriate part of the TLC adsorbent (examined under UV light) was extracted with methanol, centrifuged and the eluent was evaporated to dryness. The evaporation and TLC purification were carried out in the dark. The pale yellow oily residue (85 mg) was analysed. The isotope envelope abundance ratio showed a 98.5% deuterium exchange from 403 to 407 m/z.
GC/MS/EI+ m/z: 407 (M+) (60), 409 (M+ 37Cl isotope) (20), 376 (15) 250 (100), 236 (20), 218 (20), 171 (20), 143 (45), 113 (20), 70 (50). *GC/MS/CI- m/z:* (in methane) 407 (M-) (100), 409 (35).
^1H NMR δ : 7.15 (^1H d.) 7.11 (^1H d.) 7.01 (^1H s). Only the relevant aromatic section (6 to 8 ppm) is presented. The aliphatic section is identical with the unlabelled product.
LC/UV/DAD/MS particle beam system: Retention times for labelled and unlabelled perphenazine were 2.75 and 2.88 respectively. The lambda maxima were 258 nm and both compounds identified as perphenazine with a purity level of 998.529.

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REFERENCES

1. Previous publications: References 5 and 6.

2. Werstiuk N.H. - Abstract book of the 4th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, page 83, Toronto, (1991). Published by the International Isotope Society.

3. Werstiuk N.H. and Timmins G. - Can. J. Chem. 64:1564 (1986) and other references in this journal.

4. Aulich T.R, Hawthorne, S.B., Miller, D.J. and Farnum, S.A. - The Pittsburgh Conference and Exposition, 1987 Abstracts, No:484, Atlantic City, New Jersey.

5. Koves G.J. - J. Label. Compounds Radiopharm. 24:455 (1987).

6. Koves G.J. - J. Label. Compounds Radiopharm. 29:15 (1991).

7. Abramowitch R.A. - Organic Prep. and Proced. Int. 23:683 (1991).

8. Jan-Olov Thorell, Stone-Elander, S. and Elander, N. - J. Label. Compounds Radiopharm. 31:207 (1992).

9. Stone-Elander S. and Elander N. - Abstract book of the 4th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, page 200, Toronto, (1991). Published by the International Isotope Society.

10. Stone-Elander S. and Elander N. - Appl. Radiat. Isot. 44: 889 (1993).

11. Raner, K.D., Strauss, C.R., Vyskoc, F. and Mokbel, L. - J. Org. Chem. 58:950 (1993).

12. Baba, S., Furuta, T., Sasaki, Y., and Kasuya, Y. - J. Label. Compounds and Radiopharm. 22:149 (1985) and other references in this article.