Regiospecific Deuteriation and Tritiation of Various Drugs Using a Homogeneous Rhodium Trichloride Catalyst

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Abstract □ The deuteriation and tritiation of a number of drugs containing carboxyl, amide, aralkylamine, and anilide functional groups have been investigated using homogeneous rhodium trichloride as a catalyst. Good incorporation of deuterium was observed and the regiospecificity for ortho exchange was very high for most of the drugs studied. Similarly, with tritium, good incorporation (specific activities) and regiospecificities were achieved in many cases. Satisfactory results were also obtained from the small number of heterocyclic-containing drugs that were included in the present study.

Drugs labeled with tritium or carbon-14 are widely used as research tools in the life sciences.[1] Until recently, the latter isotope has been preferred mainly because the position and stability of the label is assured. However, the need to synthesize compounds of higher specific activity than can be obtained with carbon-14 has led to a shift in emphasis.[2] This change has coincided with the development of ^3H NMR spectroscopy[3] which has provided a rapid and reliable method of assessing labeling specificity. Consequently, there is a renewed interest in the development of one-step catalytic procedures that lead to products of high specific activity, with the tritium being located in metabolically safe positions.

Catalytic reduction of a double bond or dehalogenation with tritium gas frequently offer the best routes to the preparation of a tritiated compound with a high specific activity.[4] The need to synthesize the appropriate precursor can often be a serious disadvantage however; hence, the attraction of hydrogen isotope exchange procedures, of which there are several kinds.

Compounds containing benzylic hydrogens can be specifically labeled by exchange with tritium gas over a supported palladium catalyst,[5] the procedure only being limited by the possibility of side reactions such as reduction and dehalogenation. Hydrogen isotope exchange in the presence of tritiated water and heterogeneous metal catalysts is another widely used procedure[6] which more often than not leads to a generally labeled product. However, recent studies[7-9] on both the deuteriation and tritiation of a number of substituted benzoic acids using homogeneous rhodium trichloride as catalyst shows that the label can be specifically incorporated ortho to the carboxyl group. Subsequent studies[10] have extended the range and applicability of the method so that a number of aromatic acids, amides, aralkylamines, and anilides, as well as some heterocyclic compounds, can be labeled with high regiospecificity and good incorporation. Among the compounds studied have been two drugs; that is, nedocromil sodium[11] and sodium cromoglycate.[12] The object of the present study was therefore to see whether the observations witnessed in simple aromatic compounds could be repeated on structurally more complex pharmaceuticals containing one or more ortho-directing groups.

Experimental Section

Materials—The drugs used in the present study were all obtained from Fisons Pharmaceutical Division, with the exception of zolpidem and alpidem, which were gifts from Dr. J. Allen (Laboratoires d'Etudes et de Recherches Synthelabo, France), and flecainide, a gift from Riker Industries plc, U.K.

Deuteriation and Tritiation of the Drugs—The experimental conditions employed are summarized in Table I. After reaction, the drugs were isolated by one of the following solvent extraction procedures, depending on their acidic, basic, or neutral character.

Isolation of Acidic Drugs—Procedure A—The thick-walled reaction vessel was cooled and opened, and the contents were poured into 2 mL of HCl (4 M) and extracted with ethyl acetate (3 × 5 mL). The drug was then extracted into sodium bicarbonate (5% w/v; 3 × 2 mL) before this fraction was re-acidified with 4 M HCl and re-extracted into ethyl acetate (3 × 10 mL). The ethyl acetate fraction was washed with water (2 × 2 mL), dried over anhydrous sodium sulphate, filtered, and rotary evaporated to yield the crude product.

Isolation of Basic Drugs—Procedure B—The vial contents were poured into 2 mL of KOH (1 M) and extracted into ethyl acetate (2 × 10 mL). The drug was then back-extracted into HCl (4 M, 3 × 2 mL), cooled in ice, and precipitated by the careful addition of KOH pellets, until the pH reached 11. The drug was then extracted into ethyl acetate (3 × 10 mL); this fraction was then washed with water (10 mL), dried over anhydrous sodium sulphate, filtered, and rotary evaporated. If the HCl salt of the drug was required, this was prepared by dissolving the free base in methanolic HCl, followed by rotary evaporation to give the crude HCl salt.

Isolation of Neutral Drugs—Procedure N—The contents were poured into ethyl acetate (20 mL) and washed successively with sodium bicarbonate (2 mL), HCl (3 M, 2 mL), and water (2 mL). In each case, the 2-mL fraction was re-extracted with 10 mL of ethyl acetate, and this extract added to the ethyl acetate fraction containing the drug. This latter fraction was then dried over anhydrous sodium sulphate, filtered, and rotary evaporated to leave the crude drug.

Purification and Analysis—Where necessary, the deuteriated drugs were further purified by recrystallization (Table II) and were analyzed by mass spectrometry, using a Kratos MS 30 or MS 50 spectrometer. The percentage deuterium enrichments were corrected for natural isotopic abundances by using a correction program.[13] Deuterium (^2H) and proton (^1H) NMR analyses were also performed on the deuteriated drugs using a Bruker AM-360 spectrometer.

Normally, the tritiated drugs were purified by TLC by streaking a methanolic solution of the drug onto two Merck 20 × 20-cm silica gel F254 TLC plates which had been pre-washed in methanol. After

Table I—Deuteriation and Tritiation Conditions

Condition	Deuterium Study[a]	Tritium Study[b]
$RhCl_3 \cdot 3H_2O$	0.125 mM	6–30 mg
Drug	0.25 mM	6–30 mg
Dimethylformamide	0.75 mL	30–500 μL
Isotopic water	0.75 mL	3 μL
Temperature	108 °C	108 °C
Time	18–24 h	18–24 h

[a] Deuteriated water (99.8 atom % abundance) was used. [b] Tritiated water at 50 Ci · mL^{-1} was used.

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Table II—Deuteriation of Drugs

Compound Number	Drug	% Deuterium Incorporation	Solvent Extraction Procedure	Recrystallization Solvent	Regiospecificity, % Ortho
1	Probenecid	61	A	H_2O	>98
2	Hippuric acid	98	A	H_2O	100
3	Nedocromil sodium[a]	92	A	—	~80
4	Sodium cromoglycate[a]	77	A	—	100
5	Etenzamide	98	N	H_2O	100
6	Phenacetin	94	N	EtOH:H_2O	100
7	Niclosamide	60	N	EtOH:H_2O	98
8	Sulfanitran	90	N	EtOH:H_2O	23
9	Antipyrine	95	N	Hexane	>98
10	Paracetamol	94	N	H_2O	100
11	Phenylbutazone	40	N	Hexane	100
12	Metoclopramide[b]	70	B	EtOH:H_2O	>98
13	Aminopyrine	80	B	EtOH	>80
14	Cinchocaine[b]	33	B	EtOH	<98
15	Acecainide[b]	83	B	EtOAC	100
16	Labetalol[b]	15	B	EtOH	>98
17	FPL 62064	65	N	EtOH:H_2O	~40
18	Mefenamic acid	30	N	MeOH	100
19	Phthalylsulfacetamide	dec	A	—	—
20	Phthalylsulfathiazole	dec	A	—	—

[a] Sodium salt made by addition of 1% sodium bicarbonate solution until the pH was 6.5, followed by five volumes of acetone to precipitate the salt. [b] Labeled as the HCl salt.

development, the plates were dried, and the drug was located by viewing at 254 nm and comparing the R_f value with two side spots of the unlabeled drug. The zone was scraped from the plate and eluted with methanol. After filtration, the methanol was removed by rotary evaporation to leave the purified drug (Table III). Some drugs were purified by HPLC (Spectra Physics SP 8700 pumps) using a 500 × 10-mm Partisil ODS preparative column. Detection was carried out at 254 nm using a UViolet absorbance detector (Cecil CE 212) connected to a chart recorder. Where necessary, the drug was removed from any buffer used in the HPLC solvent by solvent extraction to leave the purified drug (Table III). Analysis of the tritiated drugs was performed by ^3H and ^1H NMR spectroscopy using a Bruker WH 90 and a Bruker AC 300 spectrometer, respectively. The spectroscopic properties of all the tritium- and deuterium-labeled drugs were consistent with the expected structures and isotopic substitution patterns.

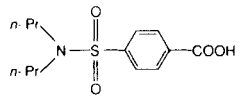
Results and Discussion

Of the 19 drugs whose deuteriation and tritiation were investigated, good isotopic incorporation together with high regiospecificity (frequently 100%) was observed in nine cases (**1**, **3**, **4**, **5**, **6**, **10**, **12**, **14**, and **15**). Moreover, a further six

Table III—Tritiation of Drugs

Compound Number	Drug	Specific Activity, mCi/mM	Solvent Extraction Procedure	TLC System	Regiospecificity, Ortho
1	Probenecid	39	A	—	>98
2	Hippuric acid	32	A	—	59
3	Nedocromil sodium[a]	80	A	*d*	100
4	Sodium cromoglycate[a]	88	A	*d*	100
5	Etenzamide	14	N	—	100
6	Phenacetin	130	N	—	>98
7	Niclosamide	—	N	—	—
8	Sulfanitran	217	N	*i*	65
9	Antipyrine	99	N	*h*	32
10	Paracetamol	42	N	*i*	100
11	Phenylbutazone	—	N	—	—
12	Metoclopramide[b]	53	B	*g*	87
13	Aminopyrine	103	B	*g*	65
14	Cinchocaine[b]	80	B	*g*	>98
15	Acecainide[b]	142	B	*g*	100
16	Labetalol[b]	—	B	—	—
17	FPL 62064	103	N	—	37
18	Mefenamic acid	123	—	*e*	51
19	Phthalylsulfacetamide	—	A	—	—
21	Flufenamic acid	114	—	*f*	51
22	Procainamide[b]	64	B	*g*	>98
23	Alpidem	654	N	*m*	57
24	Zolpidem	606	N	*l*	56
25	Flecainide[c]	34	B	*k*	100
26	Sulpiride	72	—	*j*	50

[a] Isolated as the sodium salt, as previously described. [b] Labeled as the HCl salt. [c] Labeled as the acetate salt. [d] Purified by HPLC using a gradient of methanol in 0.5% ammonium acetate solution. [e] Cyclohexane 20, ethyl acetate 30, acetic acid 2. [f] Cyclohexane 60, chloroform 30, methanol 5, acetic acid 5. [g] Methanol 60, diethylamine 1. [h] Acetone. [i] Ethyl acetate. [j] Acetone 66, *n*-butanol 30, water 3, 0.88 ammonia 1. [k] Acetone 100, triethylamine 1. [l] Chloroform 35, acetone 15, triethylamine 2. [m] Chloroform 60, triethylamine 1.



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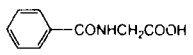
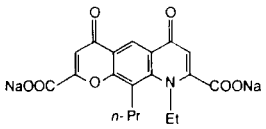


(2) HIPPURIC ACID



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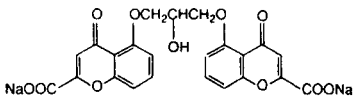
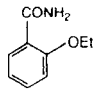


(4) SODIUM CROMOGLYCATE



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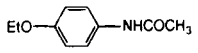

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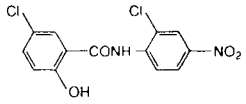

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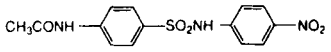
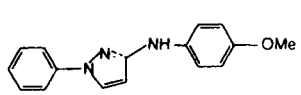


(8) SULFANITRAN



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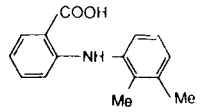

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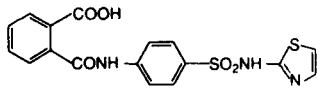
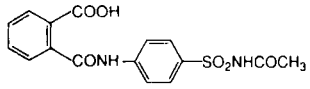
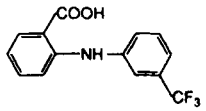


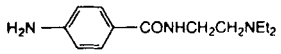
(20) PHTHALYLSULFATHIAZOLE



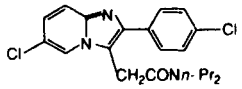
(19) PHTHALYLSULFACETAMIDE



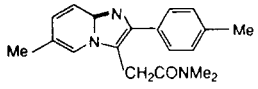
(21) FLUFENAMIC ACID



(22) PROCAINAMIDE



(23) ALPIDEM



(24) ZOLPIDEM

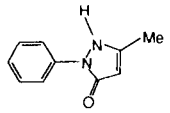
compounds (2, 7, 9, 11, 16, and 18) were deuteriated or tritiated with the same high regiospecificity. It is probable that further work to optimize the labeling conditions would provide practical deuteriation and/or tritiation routes for these agents. There were three cases (8, 13, and 17) where reduced specificity was observed and two cases (19 and 20) where decomposition occurred under the reaction conditions.

Of the six compounds (21–26) where tritiation studies alone were carried out, two (22 and 25) gave completely regiospecific incorporation, and the other four showed some labeling in sites additional to those predicted.

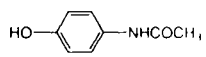
In general, the deuteriation method proved somewhat more reliable than the tritiation procedure, presumably because the catalyst is stabilized by the more aqueous solvent medium used for the deuteriation studies. Limiting cases are exemplified by 7, 11, and 16, which did not tritiate even though satisfactory deuteriation occurred. In the case of phenylbutazone (11), which is known[14] to undergo facile oxidation, the experiments were carried out in an inert atmosphere; even so, the tritiation experiments were unsuccessful. It is probable that the oxidative decomposition was promoted under the tritiation conditions.

An intermediate situation pertains when the regiospecificity of the deuteriation and tritiation procedures are different. This is the case for hippuric acid (2), where tritiation occurs in the methylene position (as well as the expected ortho position), consistent with the finding that phenylacetic is also tritiated in this position.[2] Less easy to explain are the differing results obtained for mefenamic acid (18): deuteriation occurred regiospecifically, whereas tritiation occurred equally at the expected ortho position and at the para position to the amino group, in the same ring as the carboxyl group.

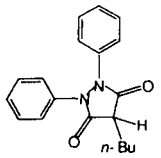
In those cases where reduced regiospecificity was observed, it was due to one of three reasons. First, the presence of electron-donating groups such as hydroxyl and amino which can promote exchange via a competing electrophilic aromatic substitution process; however, such competition was not always observed. Second, the presence of another group



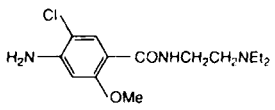
(9) ANTIPYRINE



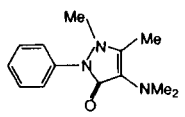
(10) PARACETAMOL



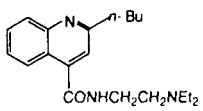
(11) PHENYLBUTAZONE



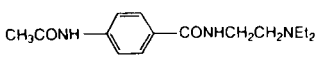
(12) METOCLOPRAMIDE



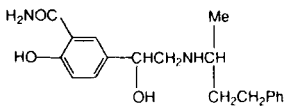
(13) AMINOPYRINE



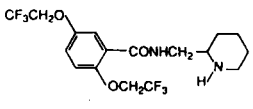
(14) CINCHOCAINE



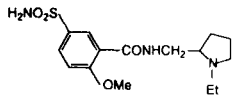
(15) ACECAINIDE



(16) LABETALOL



(25) FLECAINIDE



(26) SULPIRIDE

which, like the carboxyl group, can involve itself in a complexation–decomplexation sequence and hence direct isotope incorporation to another additional site. Thus, in the tritiation of sulfanitran (8), 35% of the tritium was in the position meta to the anilide-directing group and ortho to the sulphonamido group. A five-membered complex involving the nitrogen and the ortho ring position can be envisaged, provided that the hydrogen from the -NH group is lost. The need for a protonated nitrogen atom in such a mechanism would then explain why the fully substituted nitrogen in probenecid (1) does not lower the selectivity of the labeling promoted by the carboxyl group. Lastly, reduced regiospecificity can occur with substrates possessing activated methylene groups.

The advantages of studying both the deuteriation and tritiation reactions is illustrated in the case of acecainide (15). The mass spectrometry results show that all four ortho positions are equally labeled and consequently that equilibrium has been reached. In the tritiation studies, however, 81% of the tritium was ortho to the anilide group and the remainder ortho to the amide group, indicating that the anilide group is the more powerful ortho-directing group.

There is one reported instance[12] where the tritium distribution of one of the drugs labeled in the present study has been ascertained by classical degradation studies. When comparison is made with our ^3H NMR results (4), good agreement is obtained. Moreover, when tritiated water of >90 atom % abundance was used to label two drugs (3 and 4), the products had specific activities of 21.5 and 16.5 $Ci \cdot mM^{-1}$, respectively.[11,12] Clearly, this one-step catalytic procedure with its ability to produce regiospecifically tritiated products at such high specific activities significantly extends the range of methods for the synthesis of tritium-labeled pharmaceuticals.

Conclusions

The early work[7–9] on homogeneous hydrogen isotope exchange with rhodium trichloride as catalyst concentrated on using aromatic carboxylic acids as substrates. More recently,[10] compounds containing a wider range of directing groups have been employed. The present study has shown that when these same directing groups are present as part of a more complex structure, the same high regioselectivity and good isotope incorporation features are retained.

References and Notes

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