LABELLED COMPOUNDS OF POTENTIAL BIOLOGICAL INTEREST

II.- APPLICATION OF THE YAVORSKY METHOD FOR TRITIATION *

Andrija TELC**, Björn BRUNFELTER*** and Tamas GOSZTONYI****.

Research and Development Laboratories,
Astra Läkemedel AB,
S-151 85 SÖDERTÄLJE,
SWEDEN.

Received on March 5, 1971.

SUMMARY

The application of the Yavorsky method for tritiation of a number of aminoacetanilides, 2-amino-1-phenylpropane derivatives and some other compounds is described and briefly discussed.

Tritiation of organic compounds with the boron trifluoride complex of tritiated phosphoric acid was first published 1962 by Yavorsky and Gorin (1, 2, 3). In spite of the simplicity and versatility of this method, there are only a few references in the literature to its application (4, 5, 6, 7).

The use of labelled compounds in modern research work on new drugs is almost inevitable. In some cases the necessary investigations can be carried out at a relatively low level of radioactivity and non-specifically labelled

---

* Part I : T. Gosztonyi, B. Carnmalm, B. Sjöberg, *Acta Chem. Scand.*, 24, 3078, (1970).

** Present address : Packard Instrument Co, 1090, Vienna, Bindergasse 5-9/1/4, Austria.

*** Present address : AB Analytica, Stockholm, Sweden.

**** To whom the correspondence should be addressed.

compounds can be used. For such purposes we have found the Yavorsky method to be particularly suitable. In the course of the last few years, a number of compounds have been labelled by this method in our laboratory. We wish now to report some of the results of these labelling experiments.

Usually no systematic investigation has been carried out in order to find the best labelling conditions for each compound. In the series of aminoacetanilide derivatives (Tables 1-3), however, a representative compound was chosen and equilibrium conditions were determined. These or similar conditions were then used throughout the series.

The theoretical percentage labelling values are calculated according to the equation given by Yavorsky (1). The number of exchangeable hydrogen positions were always considered to be equal to the total number of aromatic hydrogen positions, regardless of other possible structural influences (cf.3).

EXPERIMENTAL

Materials and methods.

The tritiating reagent was prepared according to the method given by Yavorsky (1) with a slight technical modification. The phosphorus pentoxide was covered with carbon tetrachloride in the reaction flask, and the tritiated water was added to form an upper layer. By careful shaking the water came into contact with P_2O_5. The reaction was easily kept under control by keeping the CCl_4 at a gentle reflux. After the reaction was completed, CCl_4 was removed and the tritiated phosphoric acid was saturated with BF_3.

The labelling experiments were carried out either in small screw cap polyethylene vials or in sealed glass ampoules. No agitation was used if the compound was soluble in the reagent. In cases where reaction mixtures were heterogeneous, shaking in a thermostated bath or in a drying cupboard was employed. In some experiments the compound was dissolved in a suitable solvent and the solution was shaken with the reagent. Basic compounds were usually brought into the reaction in the form of a salt (usually hydrochloride).

Recovery and purification of the labelled compounds.

After an appropriate reaction time the complex was decomposed by adding water to the reaction mixture. In labelling basic compounds the mixture was then made alkaline and extracted with ether. The compound was isolated as a salt (usually hydrochloride) from the ethereal solution. Purification was effected by recrystallization.

The purity of the products was verified by paper- or thin layer chromatography, melting point determination and IR spectroscopy.

Radioactivity of the products was measured in a Packard Liquid Scintillation Spectrometer (Models 314 EX and 3320). The chromatograms of the radioactive products were scanned by a Packard radiochromatogram scanner (Model 7200).

RESULTS AND DISCUSSION

A number of aminoacetanilide derivatives were labelled by the method. The results are summarized in Tables 1-3. As a representative α-(N-propylamino) propionanilide (Table 3 No. 1) was chosen and the labelling results were studied as a function of different parameters. Fig. 1 shows a typical curve of degree of labelling against reaction time at 50°C. These experiments showed that equilibrium is reached after 48 hours at temperatures between 50-100°C but a good practical percentage labelling can be obtained even after 24 hours.

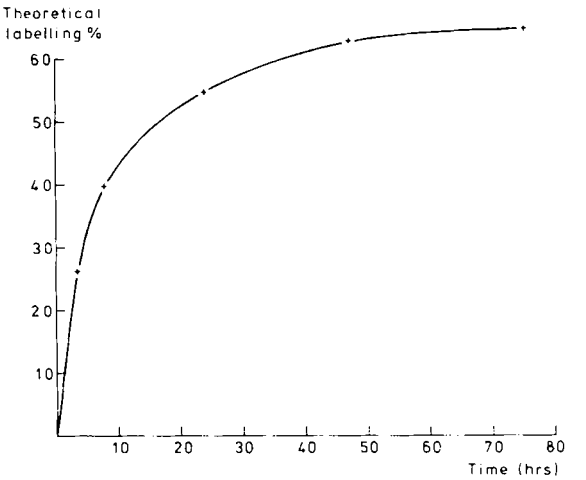


Fig. 1.

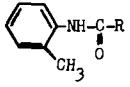
Labelling of α-(N-propylamino)-propionanilide at 50° as a function of time. (W_S/W_R = 0.15)

The ratio of the weight of the compound to that of the reagent (W_S/W_R) seemed to be of major importance. Best results could be obtained with W_S/W_R ratios between 0.1-0.3. Above 0.3 the theoretical percentage labelling values are usually lower. The compounds in this series were labelled as hydrochlorides, unless otherwise stated.

Table 4 shows the results of labelling some 2-amino-1-phenylpropane derivatives. These compounds were labelled as hydrochlorides at a relatively low activity level, except for amphetamine (No. 1).

In table 5 the results of labelling miscellaneous compounds are summarized. It can be seen that the method is applicable to a rather wide structural range. In some cases, however, either the chemical - or the radiochemical yield, or both of them were very poor (e.g. tetracaine, N-(2-chloroethyl)-dibenzylamine etc). In labelling of phenothiazines, total decomposition of the compound was usually observed. As an example, the labelling of promethazine is taken up in the table (No. 10). The product was gaschromatographically pure, but both the chemical and radiochemical yields are very poor. Our results show that the method is apparently not suitable for labelling this class of compounds.

Table 1. Aminoaceto-o-toludides

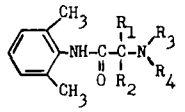


No.	COMPOUND		REAGENT		W_S/W_R	REACTION		PRODUCT			
	- R	WEIGHT W_S mg	WEIGHT W_R mg	SPEC. ACT. μCi/mg		Time hrs	Temp. °C	Chem. yield %	SPEC. ACT. μCi/mg	SPEC. ACT. mCi/mM	Theor. labelling %
1	$-CH_2-N(C_2H_5)_2$	424	2344	10.6	0.183	22	100	38	6.54	1.68	83
2	$CH_3-\underset{\vert}{CH}-NH_2$	203	391	35.8	0.519	18	90	50	1.24	0.27	5
3	$CH_3-\underset{\vert}{CH}-NH-C_2H_5$	300	2276	11.4	0.133	18	50	37	7.59	1.84	81
4[a]	$CH_3-\underset{\vert}{CH}-NH-C_3H_7(n)$	121	995	1.3	0.122	16	60	51	0.96	0.25	100
		502	3450	7.8	0.145	117	50	61	5.50	1.41	91
		816	3610	34.8	0.226	17	55	54[b]	15.90	4.08	77
5	$CH_3-\underset{\vert}{CH}-NH-C_3H_7(i)$	397	2135	11.4	0.186	22	100	45	6.00	1.54	70
6	$CH_3-\underset{\vert}{CH}-NH-C_4H_9(n)$	412	2497	11.4	0.165	22	100	10	8.07	2.18	98
7	$CH_3-\underset{\vert}{CH}-NH-C_4H_9(i)$	443	2275	11.4	0.195	22	100	68	4.65	1.26	58
8 8	$(CH_3)_2-\underset{\vert}{C}-NH-C_3H_7(n)$	477	3735	7.8	0.127	24	50	42	4.80	1.30	83
9	$(CH_3)_2-\underset{\vert}{C}-NH-$	423	2020	10.6	0.209	22	100	58	5.67	1.68	83
10	$-CH_2-CH_2-NH-C_3H_7(n)$	235	898	18.5	0.262	22	70	38	4.98	1.28	38
11	$-CH_2-CH(CH_3)-NH-C_3H_7(n)$	232	680	18.5	0.341	22	70	59	4.31	1.17	36

[a] Citanest®

[b] Labelled as free base, isolated as hydrochloride

Table 2 Aminoaceto-2,6-xylidides



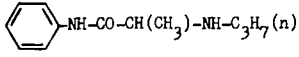
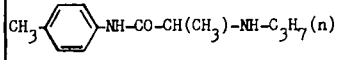
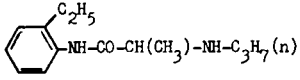
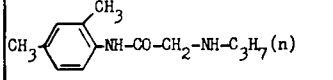
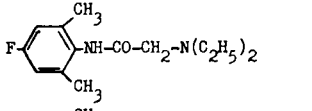
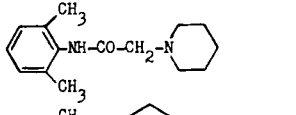
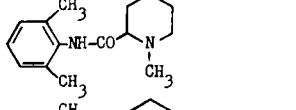
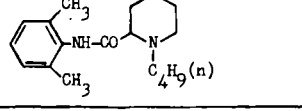
No.	COMPOUND					REAGENT			REACTION		PRODUCT			
	R_1	R_2	R_3	R_4	WEIGHT W_S mg	WEIGHT W_R mg	SPEC. ACT. µCi/mg	W_S/W_R	Time hrs	Temp. °C	Chem. yield %	SPEC. ACT. µCi/mg	SPEC. ACT. mCi/mM	Theor. labelling %
1[a]	H	H	H	H	202	412	35.8	0.492	18	90	27	7.17	1.54	36
2[a]	H	H	H	CH_3	202	596	35.8	0.339	18	90	31	7.71	1.76	36
3	H	H	H	C_2H_5	95	3536	10.4	0.027	17	50	67	5.24	1.26	78
4	H	H	H	C_3H_7(n)	134	1072	11.4	0.125	20	100	43	5.01	1.28	73
5[b]	H	H	H	C_3H_7(i)	307	1701	10.4	0.180	20	50	84	3.54	0.91	62
6	H	H	CH_3	C_4H_9(n)	300	911	5.8	0.329	90	25	65	2.40	0.68	85
7	H	H	CH_3	C_5H_{11}(n)	319	941	5.8	0.339	90	25	70	1.80	0.54	66
8	H	H	CH_3	C_6H_{13}(n)	300	866	5.8	0.347	24	60	67	2.20	0.66	85
9	H	H	CH_3	C_7H_{15}(n)	300	953	5.8	0.314	90	25	60	2.20	0.72	88
10	H	H	CH_3	$-C_4H_8OH$	251	909	7.7	0.276	23	70	17	3.00	0.90	81
11[c]	H	H	C_2H_5	C_2H_5	600	1500	32.0	0.400	68	80	51	15.90	3.72	89
12[a]	CH_3	H	H	H	198	376	35.8	0.526	18	90	22	3.15	0.72	17
13[b]	CH_3	H	H	C_2H_5	82	1186	11.4	0.068	18	50	75	5.49	1.41	77
14	CH_3	H	H	C_3H_7(n)	94	697	1.3	0.135	16	60	76	0.68	0.18	95
15	CH_3	H	H	C_3H_7(i)	275	3678	8.0	0.075	47	25	83	3.57	0.96	76
16	CH_3	H	H	C_4H_9(n)	296	1854	11.4	0.160	18	50	37	3.00	0.85	50

[a] labelling in 1 ml DMF solution

[b] labelled as the free base in 3 ml cyclohexane solution, isolated as hydrochloride

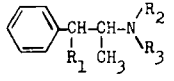
[c] Xylocaine®; labelled and isolated as free base.

Table 3 Miscellaneous aminoacetanilide derivatives

No.	COMPOUND		REAGENT		W_S/W_R	REACTION		PRODUCT			
	FORMULA	WEIGHT W_S mg	WEIGHT W_R mg	SPEC. ACT. μCi/mg		Time hr	Temp. °C	Chem. yield %	SPEC. ACT. μCi/mg	SPEC. ACT. mCi/mM	Theor. labelling %
1		91	420	1.3	0.216	16	60	76	0.69	0.17	60
2		432	1757	7.7	0.250	23	70	71	5.40	1.43	97
3		262	694	18.5	0.378	22	70	60	4.00	1.08	35
4		308	1793	11.4	0.172	20	50	57	6.92	1.83	104
5		115	940	1.3	0.122	16	60	36	0.43	0.12	93
6[a]		222	1235	8.2	0.180	72	60	80	4.5	1.05	87
7[b]		400	900	100	0.444	6	30	35	13.5	3.32	31
8[c]		300	1270	5.8	0.237	23	60	53	8.6	2.79	88

[a] Labelled and isolated as free base [b] Carbocaine®; labelled as free base, isolated as hydrochloride [c] Marcaine®

Table 4. 2-Amino-1-phenylpropane derivatives



No.	COMPOUND				REAGENT		W_S/W_R	REACTION		PRODUCT			
	R_1	R_2	R_3	WEIGHT W_S mg	WEIGHT W_R mg	SPEC. ACT. μCi/mg		Time hrs	Temp. °C	Chem. yield %	SPEC. ACT. μCi/mg	SPEC. ACT. mCi/mM	Theor. labelling %
1[a]	H	H	H	296	1181	35.8	0.250	4	90	52	28.66	5.25	73
2[b]	H	H	CH_3	203	928	5.2	0.219	12	50	52	3.04	0.56	53
3	H	H	C_2H_5	201	2030	1.0	0.099	22	90	50	0.70	0.14	58
4	H	H	C_3H_7(n)	140	1474	1.0	0.095	22	90	64	0.84	0.18	73
5	H	H	C_3H_7(i)	200	1343	1.0	0.149	22	90	54	0.22	0.05	20
6	H	H	C_4H_9(n)	200	1757	1.0	0.114	22	90	46	0.50	0.11	47
7	H	CH_3	CH_3	201	1006	3.7	0.200	66	50	43	3.51	0.70	88
8	H	C_2H_5	C_2H_5	200	2003	1.0	0.099	22	90	35	0.47	0.10	44
9[c]	OH	H	CH_3	500	4837	7.8	0.104	88	50	48	1.44	0.29	15

[a] Amphetamine [b] Deoxyephedrine [c] Ephedrine

Table 5 Miscellaneous compounds

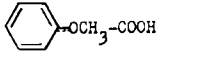
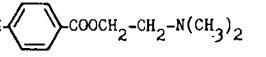
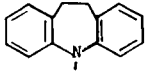
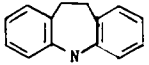
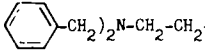
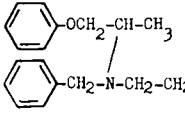
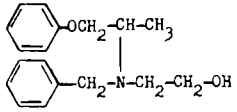
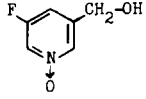
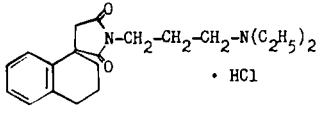
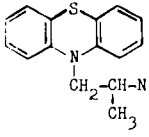
No.	COMPOUND	REAGENT			W_S/W_R	REACTION		PRODUCT			
	NAME AND FORMULA	WEIGHT W_S mg	WEIGHT W_R mg	SPEC. ACT. μCi/mg		Time hr	Temp. °C	Chem. yield %	SPEC. ACT. μCi/mg	SPEC. ACT. mCi/mM	Theor. labelling %
1	Phenoxyacetic acid 	407	1842	22.7	0.221	72	25	43	27.70	4.20	94
2	Tetracaine 	400	900	100	0.440	17	50	55	1.80	0.47	3
3	Imipramine 	385	3365	10.6	0.114	24	90	71	4.41	1.39	34
4	Desipramine 	412	2079	10.6	0.198	24	90	36	4.96	1.50	41
5	N-(2-chloroethyl)-dibenzylamine 	238	1532	18.5	0.155	22	70	36	0.04	0.12	0.1
6	Phenoxybenzamine 	849	1561	35.8	0.544	4	90	41	12.95	4.40	42

Table 5. (cont.)

No.	COMPOUND		REAGENT		W_S/W_R	REACTION		PRODUCT			
	NAME AND FORMULA	WEIGHT W_S mg	WEIGHT W_R mg	SPEC. ACT. μCi/mg		Time hr	Temp. °C	Chem. yield %	SPEC. ACT. μCi/mg	SPEC. ACT. mCi/mM	Theor. labelling %
7	Dibenzyline alcohol 	2007	5182	5.4	0.387	6	50	6	1.79	0.51	30
8	5-Fluoro-3-pyridylmethanol N-oxide 	322	3752	2.0	0.086	66	60	15	2.17	0.31	91
9	1-(γ-diethylaminopropyl)-3--spiro-(1-tetrahydronaphthyl)--succinimide hydrochloride 	600	3170	32.0	0.190	68	80	34	15.5	4.12	88
10	Promethazine 	500	1970	54.5	0.253	69	25	4	3.2	1.05	6

REFERENCES

1. Yavorsky, P.M., Gorin, E.
J. Am. Chem. Soc. 84, 1071 (1962)

2. Yavorsky, P.M., Gorin, E.
Consolidation Coal Co. Rep. NYO-9143 (1961)

3. Yavorsky, P.M., Gorin, E.
Consolidation Coal Co. Rep. NYO-10178 (1963)

4. Hilton, B.D,. O'Brien, R.D.
J. Agric. Food Chem. 12, 236 (1964)

5. Gosztonyi, T., Kovacs, A., Marton, J.
Proc. of the Second International Conference on Methods of Preparing and Storing Labelled Compounds. Euratom Brussels 1968 p. 781

6. Hamada, M., Kiritani, R.
J. Labelled Compounds 6, 187 (1970)

7. Augustin, J., Drobnica, L., Masek, F.
Radiochem. Radioanal. Lett. 2, 147 (1969)