Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 3

SYNTHESIS OF TRITIUM-LABELLED 2-(3'-CARBOXYPROPYL)-3-IMINO-6 PARA-METHOXY PHENYL-2,3-DIHYDROPYRIDAZINE ([^{3}H] SR95531).

A. Melikian GH*, G. Schlewer*, S. Hurt+, D. Chantreux† and C.G. Wermuth*

* *Laboratoire de Chimie Organique (UA 501 du CNRS), Faculté de Pharmacie, B.P. 10, 67048 Strasbourg Cedex, FRANCE.*

\+ *DuPont, NEN Products, 549 Albany St., Boston, MA 02118, U.S.A.*

† *SANOFI-Recherche, Rue du Prof. J. Blayac, 34082 Montpellier Cedex.*

SUMMARY

The synthesis of tritium labelled 2-(3'-carboxypropyl)-3-imino-6-para-methoxyphenyl-2,3-dihydropyridazine, a new, specific, water-soluble and chemically stable GABA antagonist at the $GABA_A$ receptor site is reported. The synthesis involves an N-2 endo-alkylation of 3-amino-6-p-methoxyphenyl-pyridazine with ethyl γ-bromocrotonate, reductive tritiation of the crotonic double bond and hydrolysis in acidic medium.

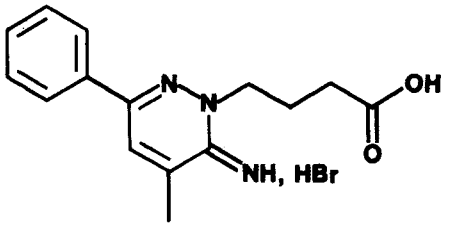
Key words : SR 95531, $GABA_A$ receptor antagonist, 2-(3'-carboxypropyl)-3-imino-6 para-methoxy phenyl-2,3-dihydropyridazine.

INTRODUCTION

Bicuculline, an alkaloïd from plants of the genera Corydalis and Dicentra was recognized in the early 1970s as an antagonist of the neurotransmitter GABA (1). Bicuculline is now considered as the most

0362-4803/87/030267–08$05.00
© 1987 by John Wiley & Sons, Ltd.
Received April 15, 1986

selective $GABA_A$ antagonist although it has been criticized for being neither potent (2) nor reliable enough (3-7). Some of the shortcomings might be due to the hydrolysis of bicuculline in aqueous solutions and to its rapid conversion to the less active hydroxy acid bicucine (8). Experiments performed with the more stable and soluble quaternary salts of bicuculline gave more consistent results (9,10). Of the more recently described $GABA_A$ antagonists, 5,6,7,8-tetrahydro-4-isoxazolo(3,4-d) azepin-3-ol (iso-THAZ) (11) also blocks neuronal inhibition elicited by glycine (12), the steroïd derivative R 5135 strongly interacts with benzodiazepine and glycine receptors (13), and pitrazepine interacts with benzodiazepine receptors (14). Very recently, two new and chemically different selective $GABA_A$ receptor antagonists have been described ; securinine which is an alkaloïd extracted from the shrub Securinega suffructicosa (15), and SR 95103 which is a synthetic GABA derivative prepared by our group (16). Both compounds appear to be relatively specific for the $GABA_A$ receptor, however securinine is somewhat weaker than bicuculline in displacing tritiated GABA from its receptor in vitro (15) whereas SR 95103 exhibits an approximately 20-fold greater affinity than bicuculline for the $GABA_A$ receptor (16). The chemical structure of SR 95103 (Fig. 1) is characterized by a GABA molecule attached at its N-terminus to a phenyl-amino-pyridazine heterocycle.



SR 95103

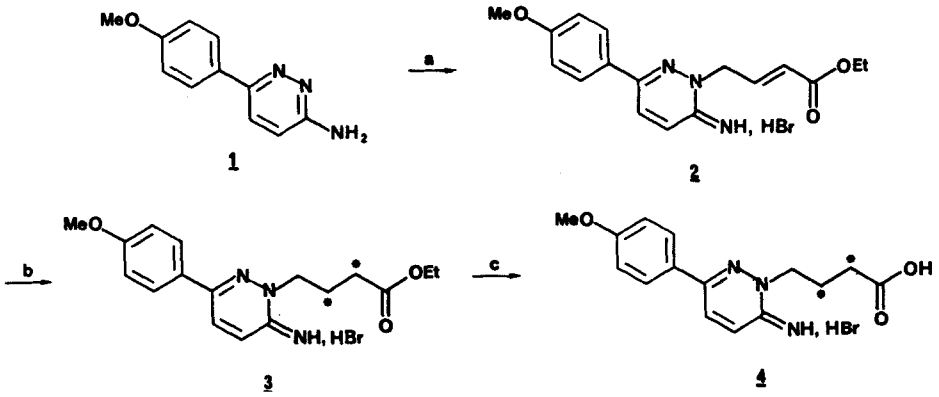
Figure 1

Structure-activity studies show that removing the methyl group at the 4-position and placing a methoxy group at the 6-position led to the compound, SR 95531, which exhibits the highest affinity in this series

and which was approximately 250 times more potent than bicuculline in displacing tritiated GABA from its receptor site (17). SR 95531 shows a very high selectivity for the $GABA_A$ receptor site (17, 18), it is freely soluble in water and chemically stable. We report here the synthesis of SR 95531 labelled with tritium on the butyric side chain. This compound may constitute a useful research tool in neurochemistry.

RESULTS AND DISCUSSION

The synthesis of [^{3}H] SR 95531 [2-(3'-carboxy- ^{3}H-2',3'-propyl-3-imino-6- para-methoxyphenyl-2,3-dihydropyridazine] was performed following the published synthesis of the unlabelled compounds of this series (16). The only modification was the replacement of ethyl 4-bromobutyrate with ethyl 4-bromocrotonate at the alkylation step, the crotonic double bond allowing the reductive tritiation (Scheme I).



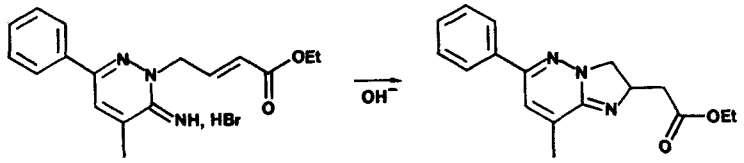
1 2 3 4

Scheme I : Synthesis of ^{3}H SR 95531

a : $Br-CH_2-CH=CH-CO_2Et$-DMF ; b : $^3H_2$-Pd/C ; c : AcOH-HCl, Zorbax ODS

Thus the alkylation of 3-amino-6-para-methoxyphenyl-2,3-dihydropyridazine 1 with ethyl 4-bromocrotonate in the minimal amount of dimethylformamide yielded the hydrobromide of 2-(3'-carbethoxypropen-2-yl)-3-imino-6-para-methoxyphenyl-2,3-dihydropyridazine 2. The synthesis was continued with this hydrobromide, without isolation of the corresponding free base. During preliminary studies in which ethyl 4-bromocrotonate was

reacted with 3-amino-4-methyl-6-phenylpyridazine, we observed a spontaneous cyclisation of the alkylation product 5 (Scheme II) after sodium hydrogen carbonate treatment and on standing in aqueous solution. The imidazolino-pyridazine 6 was formed as a result of an intramolecular Michaël addition.



5 6

Scheme II : Cyclisation into imidazolino-pyridazines

For this reason the ethylenic hydrobromide 2 was directly hydrogenated with tritium in the presence of 10 % Pd/C and the resultant saturated ester 3 was then hydrolyzed in an acetic acid-hydrochloric acid mixture.

EXPERIMENTAL

Material and procedures

3-Amino-6-para-methoxyphenyl-pyridazine 1 and 3-amino-4-methyl-6-phenyl- pyridazine 5 were prepared according to ref. (16) by hydrogenolysis of the corresponding 3-hydrazino-pyridazine (19). Melting points were taken on a Mettler PFM apparatus and are uncorrected. ^{1}H-NMR spectra were performed with a Bruker 60 MHz instrument using tetramethylsilane as internal standard.

1. 2-(3'-Carbethoxy-propen-2'-yl)-3-imino-6-para-methoxyphenyl-2,3-dihydropyridazine hydrobromide 2. To a solution of 300 mg (1.48 mmol) of 3-amino-6-para-methoxyphenyl-pyridazine in 5 drops of DMF, 315 mg (1.64 mmol) of ethyl 4-bromocrotonate were added and the mixture was heated at 60°C for 3.5 h. After cooling the precipitate was collected by filtration and recrystallized from methanol with addition of anhydrous ether. A

white powder (260 mg ; 45 %), melting at 191°C was obtained. Rf (n-BuOH-AcOH-H_2O-4:1:1) : 0.75. ^{1}H-NMR (DMSO d_6) : 1.16 (t, J = 7.0, 3H, $-CH_2-CH_3$), 3.83 (s, 3H, $-OCH_3$), 4.10 (q, J = 6.0, 2H, $-N-CH_2-$, X_2 part of an ABX_2 system), 5.8-6.2 (m, A part of an ABX_2 system, part B masked, 1H-CH=CH-CO_2Et), 6.8-8.9 (m containing (a) at 7.51 : $(AB)_2$, δ = 0.82, J_{AB} = 7.0, 4H, $-C_6H_4-$; (b) at 8.00 : AB, δ = 0.70, J_{AB} = 7.0, 2H, $C_4H_2N_2$) and (c) : a m, 1H, $-H_2C-CH=C-$), 9.16 (broad s, 2H, exch. D_2O, NH_2). Anal. calc. for $C_{17}BrH_{20}N_3O_3$: C : 51.78, H : 5.11, N : 10.65. Found : C : 51.52, H : 4.94, N : 10.83.

2. Tritium labelling : 2-(3'-carbethoxy-^{3}H-2',3'-propyl)-3-imino-6-para-methoxyphenyl-2,3-dihydropyridazine hydrobromide 3. A mixture of 10 mg (0.025 mmol) of the unsaturated precursor 2 and 10 mg of 10 % Pd/C in 5 ml of absolute EtOH was stirred for 1 hr at room temperature under 100 Curies of tritium gas at 1 atm pressure. The catalyst was removed by filtration to yield 770 mCi of crude product. The material was purified by preparative tlc on two 250 micron silica gel GF plates using CH_2Cl_2-CH_3OH (4:1) as eluent. The product (375 mCi) was 97 % radiochemically pure in the above tlc system.

3. 2-(3'-Carboxy-^{3}H-2',3'-propyl)-3-imino-6-para-methoxyphenyl-2,3-dihydropyridazine 4. A solution of 375 mCi of 3 in 0.2 ml of glacial acetic acid and 0.025 ml of concentrated HCl was heated at 100°C for 2 hrs. The reaction mixture was cooled to room temperature and then evaporated to dryness under reduced pressure. The residue was purified by HPLC on a Zorbax ODS column (4.6 mm x 25 cm) with 0.01 M NH_4OAC:CH_3CN, 7:3 as solvent. The mobile phase was removed by rotary evaporation at room temperature and the product was redissolved in absolute EtOH to give 240 mCi of product. The radiochemical purity was 98 % by tlc on silica gel GF (n-butanol-acetic acid-water, 25:4:10) and HPLC on Zorbax ODS (0.01M KH_2PO_4 pH3-CH_3CN, 7:3). Specific activity was determined by UV to be 38.2

Ci/mmole. The product was stored at 1.0 mCi/ml in absolute ethanol at -20°C.

4. 2-(3'-Carbethoxy-propen-2'-yl)-3-imino-4-methyl-6-phenyl-2,3-dihydropyridazine 5. A mixture of 500 mg (2.70 mmol) of 3-amino-4-methyl-6-phenyl-pyridazine, 575 mg (2.97 mmol) of ethyl 4-bromocrotonate and 5 ml of DMF was heated at 60°C for 2.5 h. After cooling the solid was collected by filtration and washed with anhydrous ether. A white powder was obtained (630 mg, 61 %) ; m.p. 187°C. Rf (n-BuOH-AcOH-H_2O-4:1:1) : 0.46. ^{1}H-NMR (DMSO d_6) : 1.20 (t, J = 7.5, 3H, $-CH_2-CH_3$), 2.45 (broad s, 3H, $-CH=CH-CH_3$), 4.12 (q, J = 7.5, 2H, $-CH_2-CH_3$), 5.25 (broad d, X_2 part of an ABX_2, J_{BX} = 5.3, 2H, $-CH_2-CH=CH-$), 6.55 (AB part of an ABX_2, $\Delta\delta$ = 1.14, J_{AB} = 16.0, J_{BX} = 5.3, $-CH_2-CH=CH-$), 7.4-8.2 (m, 5H, C_6H_5), 8.50 (broad s, 1H, pyridazine CH in 5-position), 9.00 (broad s, 2H, exch. D_2O, NH_2). Anal. calc. for $C_{17}BrH_{20}N_3O_2$: C : 53.97, H : 5.30, N : 11.10. Found : C : 53.95, H : 5.46, N : 10.93.

5. 2-carbethoxymethyl-6-phenyl-8-methyl-imidazolino (3,2-b) pyridazine 6. A solution of 50 mg (0.13 mmol) of the unsaturated ester 5 in 5 ml distilled water was kept at room temperature for 24 h. After removal of the solvent under reduced pressure a white powder was obtained (40 mg), m.p. 172°C. Rf (n-BuOH-AcOH-H_2O, 4:1:1) : 0.48. ^{1}H-NMR (DMSO d_6) : 1.20 (t, J = 7.5, 2H, $-CH_2-CH_3$), 2.45 (broad s, 3H, $-CH_3$ at the 8 position), 3.00 (broad d, J = 4.5, 2H, $-CH_2-CO_2Et$), 4.12 (q, J = 7.5, 3H, $-CH_2-CH_3$), 4.5-5.1 (m, 3H, $-CH_2-CH-CH_2-CO_2Et$), 7.4-8.2 (m, 5H, C_6H_5), 8.38 (broad s, 1H, -CH at the 7 position). Anal. calc. for $C_{17}BrH_{20}N_3O_2$: C : 53.97, H : 5.30, N : 11.10. Found : C : 54.11, H : 5.36, N : 11.11.

ACKNOWLEDGMENTS

The authors are indepted to Marlyse Wernert for her excellent secretarial assistance.

REFERENCES

1. Curtis D.R., Duggan A.W., Felix D. and Johnston G.A.R., Nature, 226, 1222 (1970).
2. Woodbury D.M., Convulsant drugs : Mechanisms of action. In G.H. Glaser, J.K. Penry and D.M. Woodbury (Eds). "Antiepileptic drugs, Mechanisms of Action". Raven Press, New York, 1980, pp 249-303.
3. Godfraind J.M., Krnjevic K. and Pumain R., Nature, 228, 675 (1970).
4. Curtis D.R., Duggan A.W., Felix D. and Johnston G.A.R., Nature, 228, 676 (1970).
5. Straughan D.W., Neal M.J., Simmonds M.A., Collins G.G.S. and Hill R.G., Nature, 233, 352 (1971).
6. Biscoe T.J., Duggan A.W. and Lodge D., Comp. F. Gen. Pharmacol., 3, 423 (1972).
7. Goldinger A. and Müller W.E., Neurosci. Lett., 16, 91 (1980).
8. Olsen R.W., Ban M., Miller T. and Johnston G.A.R., Brain Research, 98, 383 (1975).
9. Johnston G.A.R., Beart P.M., Curtis D.R., Game C.J.A., Mc Culloch R.M. and Maclachlan R.M., Nature, 240, 219 (1972).
10. Collins J.F. and Hill R.G., Nature, 249, 845 (1974).
11. Arnt J. and Krogsgaard-Larsen P., Brain Research, 177, 395 (1979).
12. Krogsgaard-Larsen P., Hjeds H., Curtis D.R., Leak J.D. and Peet M.J., J. Neurochem., 39, 1319 (1982).
13. Hunt P. and Clements-Jewery S., Neuropharmacology, 20, 357 (1981).
14. Gähwiller B.H., Maurer R. and Wuthrich H.J., Neurosci. Lett., 45, 311 (1984)
15. Beutler J.A., Karbon E.W., Brubaker A.N., Malik R., Curtis D.R. and Enna S.J., Brain Research, 330, 135 (1985).
16. Chambon J.P., Feltz P., Heaulme M., Restlé S., Schlichter R., Bizière K. and Wermuth C.G., Proc. Natl. Acad. Sci. USA, 82, 1832 (1985).
17. Bourguignon J.J., Schlewer G., Melikian A., Chantreux D., Molimard J.C., Heaulme M., Chambon J.P., Bizière K. and Wermuth C.G., The Pharmacologist, 27, 518 (1985).

18. Heaulme M., Chambon J.P., Leyris R., Molimard J.C., Wermuth C.G. and Bizière K., Brain Research, in press.

19. Leclerc G., Wermuth C.G., Miesch F. and Schwartz J., Eur. J. Med. Chem., 11, 107 (1976).