SYNTHESIS OF LABELLED BIOACTIVE COMPOUNDS

III. 2-(Arylimino)-imidazolidine labeled with ^{14}C, Tritium and Deuterium. *)

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SUMMARY

Five compounds of a group of 2-(arylimino)-imidazolidines, among them Clonidine (St 155), have been labeled with ^{14}C in the following manner: Potassium cyanide-[^{14}C] was converted to potassium thiocyanate-[^{14}C] and subsequently to the N'-phenylthiourea using benzoyl chloride and the corresponding aniline. The thiourea was saponified and reacted with methyl iodide to give the quaternary isothiourea derivative. Ring closure to the imidazolidine was achieved through condensation with ethylenediamine. - Tritium labeled Clonidine was obtained by catalytic debromination of the 4-bromo-derivative with tritium gas. - The starting material for deuteroclonidine was ethylene-[$^{2}H_4$]-diammonium dichloride.

ZUSAMMENFASSUNG

Fünf Verbindungen aus der Reihe der 2-(Arylimino)-imidazolidine, darunter Clonidin (St 155), wurden mit ^{14}C auf folgendem Weg markiert: Kaliumcyanid-[^{14}C] wurde zu Kaliumrhodanid-[^{14}C] und weiter mit Benzoylchlorid und dem entsprechenden Anilin zu dem N-Benzoyl-N'-phenylthioharnstoffderivat umgesetzt. Dieses wurde verseift und das entstehende N-Phenylthioharnstoff-Derivat mit Methyljodid zu dem Phenylisothiuroniumjodid reagieren gelassen. Der Imidazolidinringschluß erfolgte durch Kondensation mit Äthylendiamin. - Tritiummarkiertes Clonidin wurde durch katalytische Debromierung des 4-Bromderivats mit Tritiumgas gewonnen. - Ausgangsmaterial für deuteriertes Clonidin war Äthylen-[$^{2}H_4$]-diammoniumdichlorid.

Key words: ^{14}C, Tritium, Deuterium, 2-(Arylimino)-imidazolidines, Clonidine, Dehalogenation.

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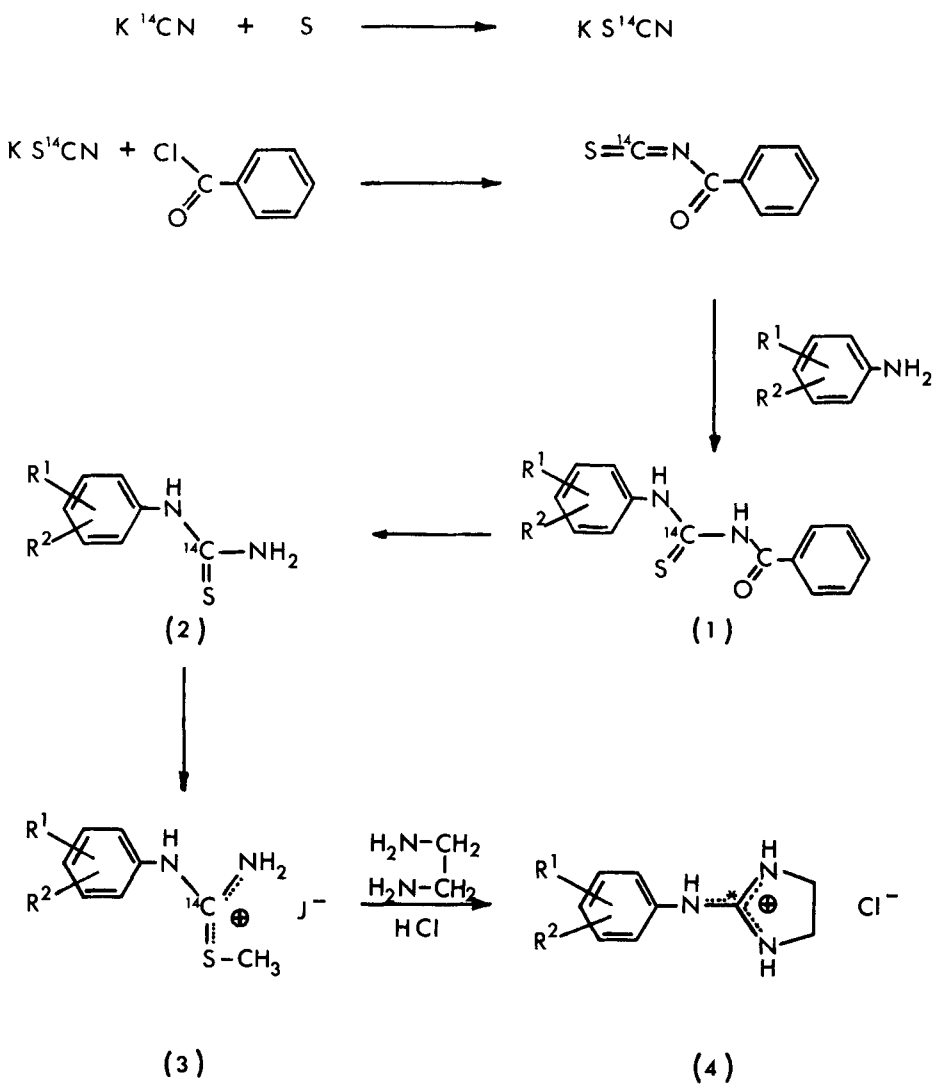
INTRODUCTION

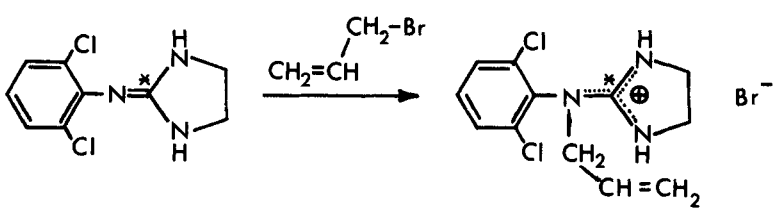
Some compounds out of the group of arylimino-imidazolidines have pronounced cardiovascular effects via stimulation of central adrenergic receptors. Best known is the hypotensive activity of Clonidine (Catapresan®), first described in 1966 [1, 2, 3]. The antihypertensive properties of the N-alkyl-derivatives, e.g. St 567 (alkyl = allyl), are reduced in favour of a strong analgesic activity. Radioactive labeling of these compounds was necessary to study biotransformation, pharmaco-kinetics and pharmacodynamics. Because of the high potency of these drugs a high specific radioactivity was desirable. Deuterated Clonidine served as internal standard for the mass spectral fragmentation [4].

DISCUSSION

The synthesis of Clonidine-[^{14}C] has been mentioned already in 1969 [5]. Some time later, Ehrhardt [6] prepared labeled Clonidine by another route, which resulted in labeling positions 4' und 5' of the imidazolidine ring with ^{14}C. We modified the earlier synthesis, increasing the radio-chemical yield more than tenfold. The preparation of N-phenyl-thiourea via the benzoyl-isothio-cyanate and N-benzoyl-N'-phenyl-isothiourea as described by Zôlyomi [7] proved to be very useful. The subsequent steps were the same as the ones published in 1966 [8].
The imidazolidine ring is usually closed in boiling methanol solution (Method A). Only the strongly sterically hindered 2.6-disubstituted derivatives have to be treated with ethylene diamine without solvent at 170 - 180^{0} (Method B).

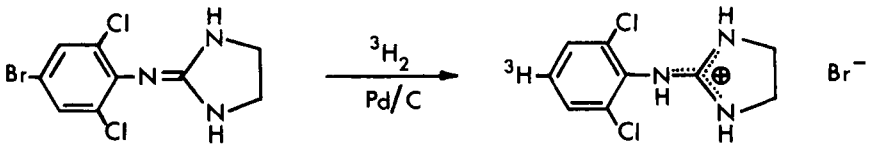


Reaction of (4) with alkyl halides results primarily in alkylation of the bridge nitrogen [10].



St 155 St 567

For the preparation of tritium labeled Clonidine we used the 4-bromo-derivative as starting material. We were aware, that on metabolic hydroxilation, which occurs predominantly in 4-position, the label would be lost; but this did not matter for the particular investigation in question [11].



For the synthesis of deuterated Clonidine etylene-[$^2H_4$]-diammonium dichloride was available. Originally we isolated the free ethylenediamine by trituration with sodium hydroxide, which resulted in some loss of material, and reacted it with (3) as shown above. Later we showed that ring closure to the imidazolidine was achieved also with the ethylenediammonium dichloride in methanolic sodium hydroxide.

EXPERIMENTAL PART

[^{14}C]-Labeled Compounds

The aryl-thioureas (2) were prepared as shown in the scheme according to [7]. Potassium cyanide-[^{14}C] was obtained from the radiochemical laboratory of Farbwerke Hoechst AG, Frankfurt or from New England Nuclear Corp., Dreieichenhain, Germany.

3.3 mMol (2) were dissolved in 5 ml methanol, 0.3 ml methyl iodide were added and the mixture was stirred with a magnet bar for 2.5 hrs. and heated to reflux. It was then concentrated in vacuo and the residue diluted with little methanol and the isothiouronium salt precipitated with ether.

Imidazolidine ring closure (Method A): 3 mMol of the 2.3- or 2.5-disubstituted arylisothiouronium salt (3) were dissolved in 10 ml methanol and 360 mg (6 mMol) ethylenediamine were added. The mixture was refluxed for 10 hours, evaporated and the residue dissolved in 2 ml methanol. After addition of 5 ml 50 % KOH the solution was extracted several times with ether. The crude base was treated with activated charcoal and recrystallized from acetone when necessary. The hydrochloride was precipitated from the methanolic solution with etheric hydrogen chloride. The hydrochlorides could be recrystallized from methanol/ether.

Imidazolidine ring closure (Method B): The 2.6-disubstituted arylisothiouronium salt (3) was immersed together with a twice molar excess of ethylenediamine in a bath of 120°. The bath temperature was raised to 180° within one half hour and the mixture kept at this temperature under stirring for 15 min. After cooling the residue was dissolved in little methanol and worked-up as described for method A.

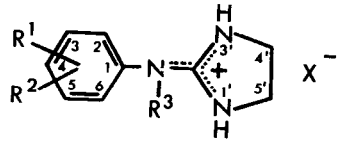
2-[N-Allyl-N-(2.6-dichlorophenyl)amino]-2-imidazoline-[2-^{14}C] hydrobromide (= St 567-[^{14}C]): The free base from 185 mg Clonidine-[^{14}C] of 14 mCi (75 µCi/mg) was liberated and transferred to an ampoule together with 85 mg allyl bromide in 2 ml acetonitrile, which was sealed. The mixture was stirred for 5 days at 60°, the ampoule opened and the contents concentrated in vacuo. The oily residue was recrystallized several times from methanol/ether. Yield 87 mg (44 % calculated for Clonidine-[^{14}C]).

[^{3}H]-Labeled Clonidine

155 mg (0.5 mMol) 2-(2.6-dichloro-4-bromophenylimino)-2-imidazolidine (free base) were dissolved in 2.5 ml tetrahydrofuran in a 5 ml flask with standard joint. A pinch of palladium-on-charcoal (10 %) was added and 1400 mg CH_3O[^{3}H] with 3 Ci were distilled into the flask in vacuo. The subsequent hydrogenation was carried out in an apparatus described by Wenzel [9], at first with 7 Ci tritium gas, later, until completion, with hydrogen. The catalyst was filtered by suction and the solvent as well as the tritium in labile postions were removed as usual. The tritiated Clonidine base was liberated from the residue, extracted with ether and precipitated as hydrochloride, which was recrystallized several times from methanol/ether. Yield 54 mg with a specific activity of 5.9 mCi/mg. By addition of non-radioactive Clonidine to the mother liquors a second crop of 115 mCi with 240 µCi/mg was obtained.

Deuterated Clonidine

A small flask was filled with 3.64 g (10 mMol) 2.6-dichlorophenylisothiouronium salt (3), 1.38 g (10 mMol) ethylene-[$^{2}H_4$]-diammonium dichloride (Huntingdon Research Centre, Huntingdon PE 18 6ES, U.K.), and 750 mg powdered sodium hydroxide suspended in 5 ml methanol. It was immersed in a bath of 120° until the methanol was evaporated. Then the temperature was raised to 180° within 20 min and kept at this temperature for additional 10 min. After cooling the brown



Code	Position and identity of				Specif. activity	Radiochemical	
	R^1	R^2	R^3	label	(mCi/mmol)	yield (%)	purity (%)
St 91	2 -C_2H_5	6 -C_2H_5	H	2'-^{14}C	51	22	> 99
St 155 *	2 -Cl	6 -Cl	H	2'-^{14}C	58	46	> 98
St 155 *	2 -Cl	6 -Cl	H	4',5'-$^{2}H_4$	-	-	-
St 155 *	2 -Cl	6 -Cl	H	4 -^{3}H	1,600	6,2	> 97
St 567	2 -Cl	6 -Cl	allyl	2'-^{14}C	20	20	> 97
St 600	2 -CH_3	5 -F	H	2'-^{14}C	32	9	> 99
St 608	2 -Cl	3 -CH_3	H	2'-^{14}C	34	37	> 99

* St 155 = Clonidine

Table of the compounds synthesized

mass was dissolved in little methanol, aquous ammonia was added and the alkaline products were extracted with ether. The ether extracts were washed several times with water and the hydrochloride precipitated as described above. Yield 1.86 g Clonidine-[^{2}H] = 70 % calculated for ethylenediammonium dichloride.

Analytical Controls

The purity of all compounds was checked by thin layer chromatography and by measuring the UV spectra. The characteristic data are given in the following table:

	Thin layer System No	R_F	$E^{1\,\%}_{1\,cm}$	
St 91	I	0,49	23,2	(264 nm)
	IV	0,50		
St 155	III	0,43	19	(271 nm)
	V	0,58		
St 567	V	0,20	19	(273 nm)
St 600	II	0,45	75,4	(273 nm)
	IV	0,38		
	VI	0,44		
St 608	II	0,52	22	(265 nm)
	V	0,39		

I. n-butanol/formic acid/water 50/5/25 (v./v.) (upper layer)

II. s-butanol/formic acid/water 75/15/10 (v./v.)

III. n-butanol/acetic acid/water 4/1/5 (v./v.) (upper layer)

IV. ethanol/acetic acid 90/10 (v./v.)

V. benzene/dioxane/ethanol/ammonia 50/40/5/5 (v./v.) (upper layer)

VI. ethylacetate/dioxane/methanol/water/triethylamine 60/30/10/10/10 (v./v.)

When necessary, the compounds were purified by column chromatography (silica with a chloroform/methanol gradient). The radio-

chemical purity was assessed by reversed dilution analysis; the identities were confirmed by comparison of mass spectra (Varian MAT CH 7).

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