Synthesis of ^{14}C-labelled 2-aminothiazole, 2-amino-5-nitrothiazole, 2-chloro-5 nitrothiazole, and 1-(5-nitro-2-thiazolyl)-2-imidazolidinone (active substance of Ambilhar®).

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SUMMARY

A description is given of the preparation of 1-(5-nitro-2-thiazolyl)-2-imidazolidinone (active substance of the antiparasitic drug AMBILHAR®) in labelled form. By introducing ^{14}C into the molecule, at position 4 of the imidazolidinone ring in one case and at position 2 of the thiazole ring in the other case, two differently labelled preparations were produced. The specific activities were 2.5 mCi/mmole and 0.33 mCi/mmole respectively. As intermediate products and by-products of the syntheses, three further derivatives of ^{14}C-thiazole which have not hitherto been described were obtained, i.e. 2-aminothiazole-2-^{14}C, 2-amino-5-nitrothiazole-2-^{14}C, and 2-chloro-5-nitrothiazole-2-^{14}C.

INTRODUCTION

1-(5-Nitro-2-thiazolyl)-2-imidazolidinone (proposed international non-proprietary name: niridazole) is the active substance of the drug AMBILHAR®, which is employed to treat patients suffering from bilharziasis and other diseases due to infestation with parasites [1, 2]. In the course of biological and clinical investigations with this drug, its metabolic behaviour has been studied in animals and man [3, 4, 5, 6, 7]. For this purpose, radioactively labelled niridazole was used, the synthesis of which is described in the following account.

SYNTHESIS.

The labelling of niridazole (VIIA, VIIB) was carried out in two different ways, each entailing the introduction of one ^{14}C atom into the molecule of the substance; in one case, the label was attached at position 4 of the imidazolidinone ring and, in the other case, at position 2 of the thiazole ring. These positions can be regarded as "metabolically secure", i.e. there is little likelihood of the C atoms in question becoming split off from the molecule in the

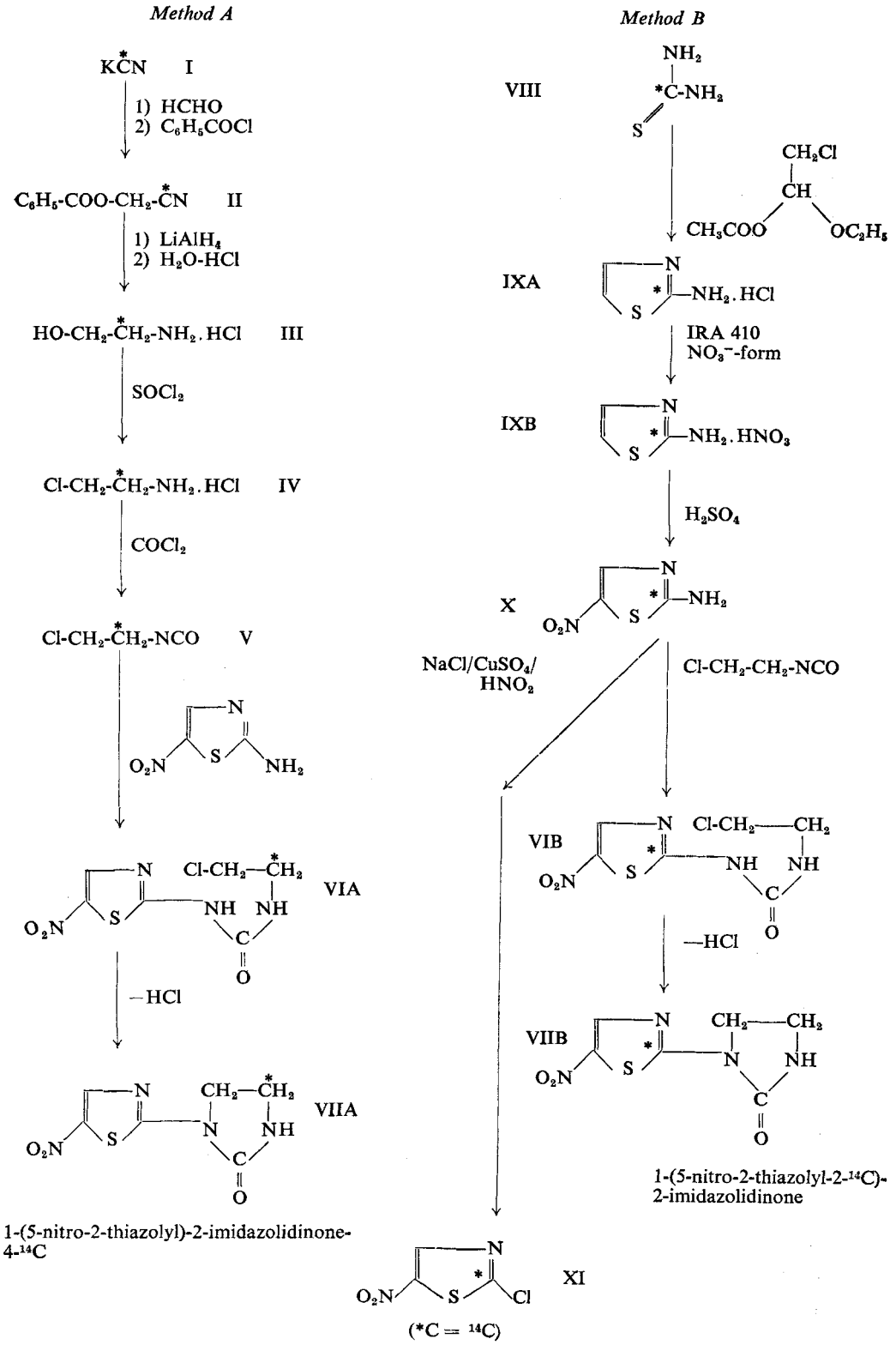
Method A
KCN I
1) HCHO
2) C6H5COCl
C6H5-COO-CH2-CN II
1) LiAlH4
2) H2O-HCl
HO-CH2-CH2-NH2.HCl III
SOCl2
Cl-CH2-CH2-NH2.HCl IV
COCl2
Cl-CH2-CH2-NCO V
VIA
—HCl
VIIA
1-(5-nitro-2-thiazolyl)-2-imidazolidinone-4-14C
Method B
VIII
CH2Cl
CH
CH3COO
OC2H5
IXA
NH2.HCl
IRA 410
NO3−-form
IXB
NH2.HNO3
H2SO4
X
NaCl/CuSO4/
HNO2
Cl-CH2-CH2-NCO
VIB
—HCl
VIIB
1-(5-nitro-2-thiazolyl-2-14C)-2-imidazolidinone
XI
(*C = 14C)

FIG. 1

form of small fragments during the course of metabolic transformations. The pathways for each of the two syntheses are outlined in figure 1. The total yield of labelled niridazole—starting with K*CN for Method A and with Ba*CO_3 for method B—amounted to approx. 28 % and approx. 24 %, respectively.

In Method B, as can be seen from figure 1, 2-aminothiazole (IXA, IXB) and 2-amino-5-nitrothiazole (X) are obtained as labelled intermediates. From the latter derivative it is also possible to produce labelled 2-chloro-5-nitrothiazole (XI). So far as we are aware, no directions for the labelling of these three compounds with radioactive carbon have yet been published in the literature. These compounds can also be employed as starting materials for other derivatives of thiazole-2-^{14}C.

Experimental part (*C = ^{14}C)

Method A.

The K*CN (I) used as the starting product for this synthesis was prepared from Ba*CO_3 by one of the known methods. The further process of conversion to Compounds II and III, as described below, represents an improvement on a labelling procedure which has already been published [8]. (For the synthesis of IV, V, VIA, and VIIA, cf. References 9 and 10.)

(Glycolonitrile-1-^{14}C)-benzoate (II).

1.95 g K*CN (30 mmole; 2.5 mCi/mmole) was dissolved in 6 ml water, mixed with 2.59 ml of a 40 % aqueous formaldehyde solution (approx. 34 mmole), and stirred magnetically for 30 minutes in an ice-bath. After the addition of 3.72 ml benzoyl chloride (4.50 g; 32 mmole), the mixture was stirred for a further 3 hours at 0° C; 10 ml of saturated aqueous sodium-bicarbonate solution was then added, after which the mixture was stirred for a further 30 minutes at room temperature and then left to stand overnight at room temperature. On the next day, the mixture was extracted with 4 aliquots of ether (30-40 ml each); the extract was dried and concentrated by evaporation in a vacuum. The oily residue crystallised following the addition of a seed crystal.

Yield : 4.83 g (glycolonitrile-1-^{14}C)-benzoate; $C_9H_7NO_2$ (100 %); m.p. 25° C (comparison preparation 27° C)*.

2-amino-ethanol-2-^{14}C-hydrochloride (III).

4.83 g (30 mmole) of Compound II was dissolved in 20 ml of distilled, anhydrous tetrahydrofurane (= T. H. F.) and — in an atmosphere of dried

* The meeting points are uncorrected

nitrogen—it was then added to a mixture of 2.52 g $LiAlH_4$ and 35 ml of distilled, anhydrous T. H. F. over a period of 1 hour, throughout which time the mixture was continually stirred and the internal temperature remained at below 30° C. The mixture was then stirred for a further hour at room temperature and for 30 minutes at reflux temperature, after which the reaction flask was cooled in an ice-bath; one after another, the following were then added drop by drop : 2.52 ml water, 2.52 ml of a solution of 1.5 g sodium hydroxide in 8.5 ml water, and, lastly, 5.04 ml water; to keep the mixture stirrable during these operations, T. H. F. was added to it. In this way an hydroxide precipitate was obtained in easily filtrable form. This precipitate was filtered off and thoroughly washed with 150-200 ml T. H. F. After 20 ml of 2N hydrochloric acid had been added to the yellowish filtrate, the latter was concentrated in a vacuum to a volume of approx. 20 ml; the resultant aqueous acid mixture was purified by extraction (repeated 3 times, using 10 ml chloroform on each occasion) and the aqueous phase evaporated in a vacuum (the bath being heated to a temperature of 80° C by the end of the process). The oily residue (2.82 g) was dissolved in approx. 10 ml ethanol, and the solution slowly mixed with 10-15 ml ether, during which operation the reaction product slowly crystallised out. A few hours later, it was sucked off, washed with an ethanol-ether mixture and with ether, and then dried in a desiccator.

Yield : 1.46 g 2-amino-ethanol-2-^{14}C-hydrochloride; $C_2H_7NO \cdot HCl$ (50 %); m.p. 81-82° C (comparison preparation 82.5-84° C).

When the above procedure was repeated, using the same quantities of materials, the yield of Compound III was 1.50 g. For further processing, this amount was pooled with the 1.46 g previously obtained.

2-chloro-ethylamine-1-^{14}C-hydrochloride (IV).

2.96 g (30.4 mmole) of Compound III was mixed with 15 ml of anhydrous toluene and 3.07 ml (5.0 g; 42 mmole) thionyl chloride and stirred magnetically in a closed flask for 18 hours at room temperature and then for 1 hour at 50° C. After the mixture had been cooled to room temperature, the crystalline reaction product was filtered off, washed with toluene and benzene (approx. 10 ml each), and dried at 50° C in a high vacuum.

Yield : 3.44 g 2-chloro-ethylamine-1-^{14}C-hydrochloride; $C_2H_6ClN \cdot HCl$ (98 %); m.p. 142-148° C [unpurified product; pure comparison preparation [9] : 147-148° C].

(2-chloro-ethyl-1-^{14}C)-isocyanate (V).

A suspension of 3.44 g (29.6 mmole) of Compound IV in 6.7 ml of distilled 1,2,4-trichlorobenzene was stirred magnetically for 3 hours at a temperature of 138-140° C under exclusion of humidity. During this time, a stream of

phosgene was conducted through the apparatus by means of a gas inlet-tube, the end of which was placed just above the surface of the fluid. After the 3 hours had elapsed, the resultant brownish solution was cooled to room temperature, and the excess phosgene dissolved in it was removed by a 5-minute period of evacuation, using a water-jet pump. This solution of (2-chloro-ethyl-1-^{14}C)-isocyanate, C_3H_4ClNO, was employed for the next stage without having undergone any further purification.

N-(5-nitro-2-thiazolyl)-N'-(2-chloro-ethyl-1-^{14}C)-urea (VIA) and 1-(5-nitro-2-thiazolyl)-2-imidazolidinone-4-^{14}C (VIIA).

The solution of Compound V (theoretical quantity : 29.6 mmole) in trichlorobenzene, which had been obtained from the previous reaction, was added over a period of 20 minutes to a stirred mixture of 4.35 g (30 mmole) 2-amino-5-nitrothiazole [10], 1.50 g of powdered, anhydrous potassium carbonate, and 21 ml acetone. While this addition was being made, the mixture was warmed to a temperature of 40-50° C. The dropping funnel was rinsed with 6-8 ml acetone and the mixture stirred for 2 hours at 45-50° C, after which the acetone was evaporated in a vacuum at approx. 40° C. The resultant intermediate product, i.e. N-(5-nitro-2-thiazolyl)-N'-(2-chloro-ethyl-1-^{14}C)-urea, $C_6H_7ClN_4O_3S$, was not isolated.

The concentrated mixture, which still contained the high-boiling solvent trichlorobenzene, was mixed with 6 ml N,N-dimethylformamide (= D.M.F.) and 4.10 g sodium acetate ($CH_3COONa.3H_2O$; 30 mmole). The reaction flask was immersed in a bath pre-heated to a temperature of 85-90° C, and the mixture was then stirred for 30 minutes at this temperature, after which it was concentrated in a vacuum and the remnants of the high-boiling solvents were removed in a high vacuum at a temperature of 100° C. The brown residue was mixed with 6 ml D.M.F. and 25 ml water, and the mixture was left to stand for several hours at 0° C. The brown crystalline precipitate formed was sucked off and successively washed with approx. 5 ml each of water, methanol, ethanol, and petroleum ether and finally dried in a desiccator.

Yield : 4.80 g of brown raw product VIIA. By working up the mother liquor, a second fraction of 0.10 g was obtained.

Purification of the raw product VIIA : 4.90 g of the crystalline substance was dissolved in 80 ml D.M.F., mixed with 80 ml ethyl acetate, and poured on to an aluminium-oxide column (acidic, activity I; dimensions : 3.5 × 30 cm). Separation into a main yellowish zone and into several more slowly moving dark-brown and reddish-brown zones occurred in response to development with a 1 : 1 mixture of D.M.F. and ethyl acetate. The main zone was eluted in several fractions, and those fractions which thin-layer chromatography (silica gel; benzene-acetone 7 : 3) showed to be not yet quite pure were purified by carrying out a second column chromatography on aluminium oxide (acidic, activity I; eluant : D.M.F.-chloroform 1:9). The pure fractions

from both chromatographs were pooled and concentrated by evaporation, the weight of the resultant dry residue being 3.85 g. This product was dissolved in a small volume of heated D.M.F. and induced to crystallise by adding ethanol; a few hours later, the crystals were sucked off, washed with ethanol, and dried in a high vacuum at 120° C.

Yield : 3.62 g 1-(5-nitro-2-thiazolyl)-2-imidazolidinone-4-^{14}C; $C_6H_6N_4O_3S$ (57 % based on Compound IV); m.p. 262-264° C (decomp.); [pure comparison preparation : 262-264° C (decomp.)]. Purity : thin-layer chromatography (silica gel; benzene-acetone 7 : 3) revealed that the product was uncontaminated and identical with the comparison preparation [10]. Radiometric evaluation of the chromatogram showed a degree of purity of $>$ 99.5 %. Specific radioactivity : 11.8 μCi/mg, corresponding to 2.5 mCi/mmole.

METHOD B.

The ^{14}C-thiourea (VIII) employed as the starting product for this method was prepared from Ba*CO_3 via Ba*CN_2 according to known methods [11, 12], the yield being approx. 70 %. (For the syntheses of the remaining compounds, cf. References 13, 10 and 14.)

2-aminothiazole-2-^{14}C-hydrochloride (IXA).

1.06 g (14.0 mmole; 0.33 mCi/mmole) of ^{14}C-thiourea was mixed with 2.35 g (14.1 mmole) of (1-ethoxy-2-chloro-ethyl)-acetate [12] and 3 ml water and heated to 55° C while being stirred. One drop of concentrated hydrochloric acid was added and the mixture stirred for 45 minutes at the aforementioned temperature. After about 15 minutes the mixture, which had originally been in two phases, became homogeneous. At the end of the 45-minute period, it was evaporated to dryness in a vacuum at approx. 20° C. The colourless crystalline residue of 2-aminothiazole-2-^{14}C-hydrochloride, $C_3H_4N_2S \cdot HCl$, was employed for the next stage without having been purified.

2-aminothiazole-2-^{14}C-nitrate (IXB).

The dry residue IXA (theoretical quantity : 14.0 mmole) was dissolved in 4 ml water, filtered over a column of anion-exchange resin (2.4 × 17 cm; AMBERLITE IRA 410, nitrate form), and eluted with approx. 400 ml water. The filtrate was evaporated in a vacuum at 25-30° C, and the residue dried in a dessicator. The raw crystalline product thus obtained —2-aminothiazole-2-^{14}C-nitrate, $C_3H_4N_2S.HNO_3$— was used in the following reaction without having been purified.

2-amino-5-nitrothiazole-2-^{14}C (X).

The dry residue IXB from the previous step (theoretical quantity : 14.0 mmole) was cooled in a cold bath to —30° C and then, drop by drop and stirring continuously, was mixed with 7 g of similarly cooled concentrated sulphuric acid (density = 1.84). Under exclusion of humidity the suspension was now warmed from —30° C to 0° C, and its temperature was then kept at 0-5° C until all the solid particles had been completely dissolved (approx. 1 hour), after which the solution was stirred at room temperature for a further 3 hours. It was subsequently cooled to —15° C and mixed, over a period of 45 minutes, with 14 ml of an aqueous sulphamic-acid solution (1.6 mg/ml). During this operation, the temperature was allowed to rise to —5° C, whereupon at this temperature 13.5 ml of concentrated aqueous ammonia solution was added within 1 hour. The weakly alkaline mixture was extracted with ethyl acetate, and the extract dried with sodium sulphate and reduced in a vacuum to a volume of approx. 50 ml .After addition of Norit, the concentrated extract was filtered over a silica-gel column ("Davison", 2.4 × 15 cm), eluted with a total of 500 ml ethyl acetate, and evaporated to dryness in a vacuum.

Yield : 1.0 g 2-amino-5-nitrothiazole-2-^{14}C; $C_3H_3N_3O_2S$ (50 % based on thiourea); m.p. 190-198° C (decomp.); [pure comparison preparation [10] : 194-198° C (decomp.)]. Purity : no impurities visible in the thin-layer chromatogram (silica gel; toluene-acetone 7 : 3); identical with the comparison preparation. The product was used in the subsequent reactions without having undergone any further purification.

N-(5-nitro-2-thiazolyl-2-^{14}C)-N'-(2-chloro-ethyl)-urea (VIB) and 1-*(5-nitro-2-thiazolyl-2-^{14}C)-2-imidazolidinone (VIIB).*

Compounds VIB and VIIB were prepared using the procedure already described for the preparation of VIA and VIIA, i.e. by means of a reaction between equimolar quantities of 2-amino-5-nitrothiazole-2-^{14}C (X) and 2-chloro-ethyl-isocyanate. However, since it was possible to use a chemically pure isocyanate as a reactant (instead of the trichlorobenzene-containing solution of the crude labelled isocyanate V), the evaporating and purifying operations at the end of the synthesis were thus simplified.

Yield : 70 % (based on 2-amino-5-nitrothiazole-2-^{14}C).

2-chloro-5-nitrothiazole-2-^{14}C (XI).

While stirring continuously, 0.90 g (6.2 mmole) of Compound X, dissolved in a mixture of 6.5 ml water and 3.5 ml of concentrated sulphuric acid (density = 1.84), was mixed first with a solution of 2.35 g sodium chloride in 7.5 ml water and then with a solution of 5.0 g copper sulphate ($CuSO_4 \cdot 5H_2O$)

in 9 ml water. At a temperature of 15-20° C and over a period of 30 minutes, a solution of 0.80 g sodium nitrite in 5 ml water was now added; after stirring for 30 minutes at room temperature, the mixture was extracted four times with a total of 100 ml ethyl acetate; the extract was then dried with sodium sulphate and concentrated to a volume of 30-40 ml. After the addition of Norit, the mixture was filtered over a silica-gel column ("Davison", 2.4 × 15 cm) and eluted with approx. 500 ml ethyl acetate. The eluted material was evaporated in a vacuum, and the yellowish crystalline residue was then dried.

Yield : 0.73 g 2-chloro-5-nitrothiazole-2-^{14}C; $C_3HClN_2O_2$ (72 %). Purity : homogeneous in the thin-layer chromatogram (silica gel; toluene-acetone 7 : 3), and identical with a comparison preparation [14]; sufficiently pure for further reactions.

A small sample was recrystallised from ether-petroleum ether. Melting point 56-58° C (pure comparison preparation : 56-58° C). Specific radioactivity : 2.0 μCi/mg, corresponding to 0.33 mCi/mmole.

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