Journal of Labelled Compounds — July-September 1965 — Vol. I, No. 3.

Synthesis of ^{14}C-labelled benzetimide-HCl *

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SUMMARY

The synthesis of ^{14}C-benzetimide (dl-1-benzyl-4-(2,6-dioxo-3-phenyl-3-piperidyl)-piperidine hydrochloride), using benzyl cyanide ^{14}C (from $K^{14}CN$ and benzyl bromide) as starting material, is described. The five steps synthesis results in an overall yield of 38 % of labeled benzetimide HCl (calculated on KCN used) with a specific activity of 0.5 mC/mM.[1] *The stability of benzetimide-HCl at 37° C with varying pH is examined.*

INTRODUCTION

Benzetimide (formerly named dioxatrine, (dl-1-benzyl-4-(2,6-dioxo-3-phenyl-3-piperidyl)-piperidine hydrochloride) was recently synthesized and its important pharmacological properties have already been described [(1)].

In order to study the metabolic fate, we synthesized ^{14}C-labelled benzetimide according to the reaction scheme (shown on p. 208).

EXPERIMENTAL AND METHODS.

A number of syntheses of benzyl cyanide is described in the literature. The best results were obtained by reacting benzyl bromide with potassium cyanide in dry ethylene glycol.

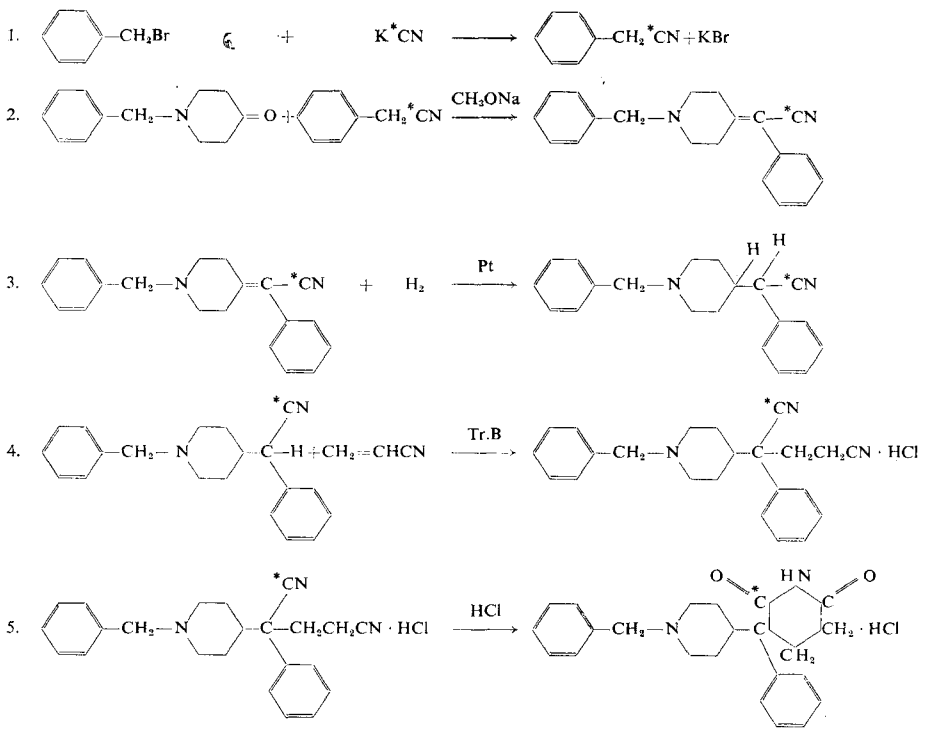
Starting with potassium cyanide-^{14}C (specific activity : 0.5 mC/mM), gas-chromatographically pure benzyl cyanide-^{14}C was synthesized. The subsequent reaction pattern was observed by means of thin-layer chromatography.

Benzyl cyanide-^{14}C was condensed with 1-benzyl-4-oxopiperidine [(2)]. After completion of the reaction, the total mixture was added to freshly hydrogenated Adams catalyst and hydrogenated at atmospheric pressure and room temperature until hardly any more hydrogen was consumed. Usually, palladium is employed as catalyst in this type of reduction, since the use of platinum oxide would result in partial reduction of the nitrile group [(3)].

* Received on 28 July 1965.

[1] A seemingly preferent alternative route with the aid of ^{14}C-acrylonitrile could not be followed because of the instability of the labelled acrylonitrile (rapid polymerization).

REACTION SCHEME



Although tertiary amines containing a benzyl group readily undergo hydrogenolysis [4], debenzylation is prevented under the reaction conditions applied (Adams catalyst, 96 % ethanol and sodium methylate). The hydrogenation conditions are extremely restricted, since hydrogenation with Adams catalyst in the presence of sodium acetate or ammonia instead of sodium methylate, in a neutral or acid medium or in an anhydrous medium, results in extensive debenzylation and the formation of other products which were not identified.

When 10 % palladium on charcoal in a neutral or acid medium is used, debenzylation and hydrogenation of the double bond occur simultaneously (JANSSEN, private communication).

Purification of the hydrogenated product by column chromatography is required, since impurities (e.g. N-benzyl-4-piperidinol[1] interfere with the condensation of dl-1-benzyl-4-(α-cyanobenzyl)-piperidine) with acrylonitrile.

[1] N-benzyl-4-piperidinol has been described in the literature as an oily liquid n_D 25.1.5514 [7]. We prepared crystalline N-benzyl-4-piperidinol by hydrogenation of N-benzyl-4-oxo-piperidine under the reaction conditions described above. Hydrogen uptake was very quick. Debenzylation did not occur. After filtration of the catalyst and extraction with diethyl-

Of the various conditions for cyanoethylation, described in the literature, the use of Triton B (benzyl-trimethyl-ammonium hydroxide) as catalyst in dioxane as solvent was found to give the best results [5]. Saponification of the dl-1-benzyl-4-(1,3-dicyano-^{14}C-1-phenyl-propyl)-piperidine-HCl with concentrated HCl yield ^{14}C-labelled dl-1-benzyl-4-(2,6-dioxo-3-phenyl-3-piperidyl)-piperidine-HCl.

Chemical purity of the labelled benzetimide was ascertained by elementary analysis of inactive benzetimide, which was obtained by a cold run under the same conditions. The melting point is too high ($\sim$300° C) to be used as a criterion for purity. Radiochemical purity was examined by scanning a two-dimensional thin-layer chromatogram. The results of an analysis of the metabolic pathway will be published elsewhere.

Benzylcyanide-^{14}C.

2.61 mg of K^{14}CN (specific activity : 25 mC/mM) was diluted with 131.4 mg of inactive KCN. The total quantity of labelled KCN (specific activity : 0.5 mC/mM) was dissolved in 1.5 ml of distilled enthylene glycol.

0.245 ml (2 mM) of freshly distilled benzyl bromide was added to the solution. The reaction mixture was stirred under anhydrous conditions for 75 minutes at 60° C.

After cooling, the solution was diluted with ether and extracted twice with a saturated solution of sodium chloride. The organic phase was dried over magnesium sulfate and filtered and the ether fractionated through a Vigreux column. The residue was freed from traces of ether by warming in vacuo.

As inactive benzyl cyanide obtained by experiments under the same conditions and in the same apparatus, appeared to be gas-chromatographically pure, the labelled product was coupled with 1-benzyl-4-exopiperidine without further purification.

Yield : 199 mg of benzyl cyanide-^{14}C (85 % on K^{14}CN).

1-benzyl-4-(α-cyanobenzylidene)-piperidine.

To a solution of 199 mg of benzyl cyanide-^{14}C (1.7 mM) in 0.7 ml of absolute ethanol was added 0.321 ml of freshly distilled 1-benzyl-4-oxo-

ether and a saturated solution of sodium chloride, followed by distillation under reduced pressure, and a viscous oil was obtained (bp_{10} = 164° C) which crystallized slowly (m.p. 60.5 — 61.5° C).

Analysis :

calculated for $C_{12}H_{17}N_1O_1$:	C 75.35 %	found : C 75.5 %
	H 8.96 %	H 9.0 %
	N 7.32 %	N 7.4 %

I.R. spectrum : > C = O absent; OH, phenyl present.

piperidine[1] (b.p. = 158° C) (1.7 mM). From a solution of 5.1 g of sodium in 120 ml of absolute methanol, 0.340 ml was added to the reaction mixture.

The mixture was refluxed with stirring in an oil-bath for 90 minutes. Thin-layer chromatography showed that all of the 1-benzyl-4-oxo-piperidine had been converted into 1-benzyl-4-(α-cyanobenzylidene)-piperidine-^{14}C.

1-benzyl-4-(α-cyanobenzyl)-piperidine-^{14}C.

50 mg of Adams catalyst was reduced with hydrogen in 2 ml of 96 % ethanol. The solution of 1-benzyl-4-(α-cyanobenzylidene)-piperidine obtained in the previous step was transferred to the hydrogenation bulb with 6.5 ml of 96 % ethanol.

The mixture was shaken under hydrogen at atmospheric pressure and at room temperature.

After 43.5 ml of hydrogen had been absorbed during 4½ hours (theory : 36 ml), the catalyst was removed by filtration.

The filtrate was diluted with toluene and extracted with a saturated solution of sodium chloride. The aqueous phase was extracted twice with toluene. The organic phases were combined and dried over magnesium sulfate. After filtration, the toluene was removed in vacuo and the remaining product, an oily residue, was adsorbed on silica gel (100 mesh) and brought on top of a column of 18 g of Merck silica gel G (length of the column 33 cm, diameter 1.2 cm).

The material was chromatographed with light petroleum/ether (40 : 60) as eluent. Samples of the fractions were tested by a colour reaction with potassiumiodoplatinate (following table). The positive ones were examined by thin-layer chromatography [2]. Developing solvent : light petroleum/ether (4 : 6).

A yellow substance remained adsorbed at the top of the column, while a small greenish-yellow band moved slowly.

Fractions 10 and 11 were combined and the solvent was evaporated in vacuo to yield a slightly yellowish oil which crystallized slowly; the yield was 370 mg of dl-1-benzyl-4-(α-cyanobenzyl)-piperidine.

[1] 1-benzyl-4-oxopiperidine and pure samples of the intermediate products of benzetimide synthesis were kindly supplied by Janssen Pharmaceutica N. V.

[2] *Thin-layer chromatography.* — The chromatoplates were prepared on ribbed glass (6). Adsorbent layer : silica gel G, according to Stahl (Merck, Darmstadt). As silica gel G contains iron and other metal ions, which cause disturbances when HCl-containing developing solvents are used, the chromatoplates were predeveloped with CH_3OH : HCl : H_2O (60 : 1.5 : 2.5), dried and stored at 50° C.

Developing solvents : 1. light petroleum (b.p. 50-70° C)/ether (40 : 60);
2. *n*-BuOH/conc. HCl/H_2O (60 : 1.5 : 2.5);
3. CH_3OH;
4. $CHCl_3/CH_3OH$ + 5 %/NH_3 (10 : 1).

The spots were located by spraying with potassium iodoplatinate (0.1 ml of 10 % $PtCl_4$ solution added to 5 ml of 2 % KI solution before spraying).

fractions	ml	1-benzyl-4-(α-cyanobenzylidene)-piperidine-^{14}C	1-benzyl-4-(α-cyano-benzyl)-piperidine-^{14}C
1	55	—	—
2	2	+++	—
3	2	+++	—
4	2	++	—
5	2	++	—
6	2	+	—
7	2	+	—
8	2	±	—
9	2	±	±
10	2	—	+++
11	80	—	+++

Fractions 2 to 8, containing 52 mg of dl-1-benzyl-4-(α-cyano-benzylidene)-piperidine, can be diluted with unlabelled compound and rehydrogenated in order to obtain a less strongly labelled product.

dl-1-benzyl-4-(1-3-dicyano-1-phenyl-propyl)-piperidine-HCl-^{14}C.

A solution of 370 mg of dl-1-benzyl-4-(α-cyanobenzyl)-piperidine (1.28 mM) in 0.75 ml of analytically pare dioxane was mixed with 0.1 ml of freshly distilled acrylonitrile (1.5 mM). A drop (20 μl) of a 40 % aqueous solution of Triton B was added. After about 1 minute a strongly exothermic reaction took place. The reaction was brought to completion by heating in an oil-bath at 55° C for 8-10 minutes.

After cooling, the reaction mixture was diluted with ether and extracted three times with a saturated solution of sodium chloride. The aqueous phase was extracted once with ether.

The combined ether extracts were dried over magnesium sulfate. After filtration, the solution was concentrated to a small volume in vacuo and mixed with ether saturated with HCl gas. This operation was repeated twice after evaporation of the ether in vacuo. The dry residue crystallized from ether. As judged by thin-layer chromatography, the collected crystals appeared to be pure.

Benzetimide HCl (dl-1-benzyl-4-(2,6-dioxo-3-phenyl-3-piperidyl)-piperidine-HCl)

The dl-1-benzyl-4-(1,3-dicyano-1-phenyl-propyl)-piperidine hydrochloride obtained in the previous step was saponified by refluxing for 16 hours in 3 ml of concentrated HCl. The reaction mixture was evaporated to dryness in vacuo. Traces of water and HCl were rmoved by treatment with toluene and evapo-

ration of the toluene in vacuo. The residue was crystallised from ether. After removal of the ether, acetone was added and a fine white crystalline product was obtained.

Recrystallisation from a mixture of methanol and isopropanol yielded 300 mg of dl-1-benzyl-4-(2,6-dioxo-3-phenyl-3-piperidyl)-piperidine-HCl (0.75 mM = 38 % of theoretical overall yield (calculated on KCN)).

RESULTS.

Analysis of the cold run : $C_{23}H_{26}N_2O_2$. HCl

calculated :	C 69.24 %	found :	C 69.0 %
	N 7.02 %		N 6.9 %
	H 6.82 %		H 7.0 %

Thin-layer chromatography.

10 and 50 μg unlabelled and labelled compound yield one spot on the chromatogram.

Spectrophotometry.

UV spectra of a 2.25 mg blanc in 3 ml CH_3OH and of 2.25 mg of benzetimide ^{14}C HCl in 3 ml CH_3OH. λ_{max} = 257 mμ; = ε approx. 760.

Radiochemical purity.

After thin-layer chromatography on ribbed glass plates, the strips were scraped off in 3 mm portions. The activity of these portions was counted in a Packard Liquid Scintillation Counter 314 EX.

The silica gel was kept suspended in the liquid scintillator with Cab-O-Sil [1].

The chromatoplates showed only one spot with an Rf value identical with that of unlabelled benzetimide.

Autoradiography of a two-dimensional chromatoplate confirmed the radiochemical purity of the labelled benzetimide.

Stability of ^{14}C-benzetimide.

These experiments were carried out in order to prevent hydrolysis products of benzetimide from being considered as metabolites. Benzetimide was incubated at 37° C under sterile conditions in the following buffers :

a) Citric-phosphate (0.1 M/0.2 M) mixtures according to MCELVAINE [2], pH 2.2, 6.8, 7.4;

b) Borax/HCl (0.05 M/0.1 n) mixture according to SORENSEN [1], pH 8.6.

[1] Finely divided silicone dioxide, giving an optically clear thixotropic gel, when dispersed in the counting solution; Packard Instruments Int. S. A. Zürich, Switzerland.

After 4 and 24 hours, and again after 4 days, samples were chromatographed by thin-layer chromatography; this was followed by autoradiography and liquid scintillation scanning.

After 4 days incubation at pH 8.6 and 37° C, benzetimide is partly hydrolysed to its amide; at the other pH values benzetimide was stable.

ACKNOWLEDGEMENT

Our thanks are due to Prof. Dr H. VELDSTRA for his constant interest.

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