# SYNTHESIS OF 6-(D-α-AMINOPHENYLACETAMIDO-1-$^{14}$C)-PENICILLANIC ACID.

U. Valcavi.

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## SUMMARY

*The synthesis of 6-(D-α-aminophenylacetamido-1-$^{14}$C)-penicillanic acid (V) is described. Benzaldehyde (I) was converted with sodium cyanide-$^{14}$C and ammonium chloride into D,L-α-aminophenylacetic acid-1-$^{14}$C (II), from which the D-form was separated with D-camphorsulfonic acid. The D-α-aminophenylacetic acid-1-$^{14}$C (III) was converted, with ethyl acetoacetate into enaminoderivative (IV), which, by means of reaction with ethyl chlorocarbonate and with triethylammonium salt of 6-aminopenicillanic acid, gave 6-(D-α-aminophenylacetamido-1-$^{14}$C)-penicillanic acid(V).*

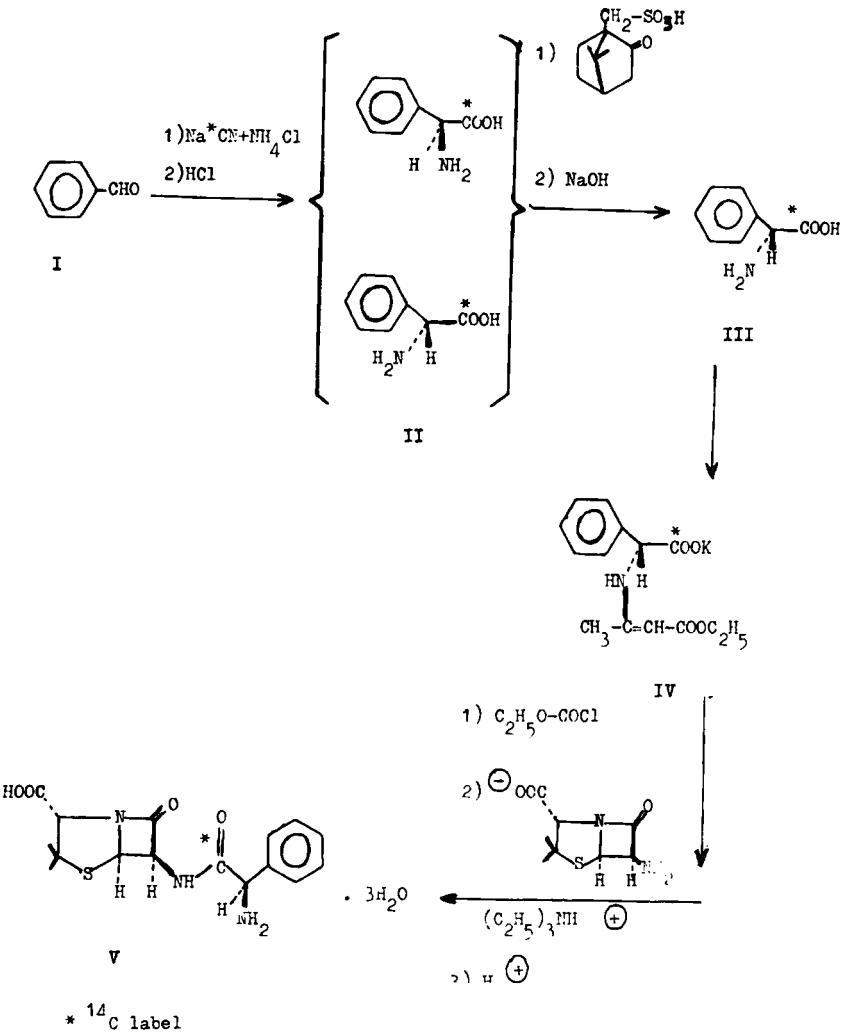
## INTRODUCTION

The 6-(D-α-aminophenylacetamido)-penicillanic acid (V), ampicillin, is one of the most useful antibiotics and one of most today used semisynthetic penicillins [1-6]. It is not till now completely known the in vivo metabolism of ampicillin and for such purpose 6-(D-α-aminophenylacetamido-1-$^{14}$C)-penicillanic acid(V) was synthetized.

## RESULTS

Benzaldehyde (I), by means of reaction with sodium cyanide $^{14}$C and ammonium chloride and then by means of hydrolysis with hydrochloric acid according [7], was converted into D,L-α-aminophenylacetic acid-1-$^{14}$C (II).

From D,L-α-aminophenylacetic acid-1-$^{14}$C (II), the D-form (III) was separated as D-camphorsulfonate [8-10]. The L-α-aminophenylacetic acid-1-$^{14}$C remaining in the mother liquor was again converted with NaOH at 100° into D,L-form from which D-α-aminophenylacetic acid (III) was again recovered, as indicated above, with D-camphorsulfonic acid.


1)Na*CN+NH4Cl
2)HCl
CHO
I
COOH
H NH2
II
1)
CH2-SO3H
O
2) NaOH
III
COOK
HN H
CH3-C=CH-COOC2H5
IV
1) C2H5O-COCl
2) OOC
(C2H5)3NH
HOOC
V
. 3H2O
* 14C label

The compound (III) was treated with ethyl acetoacetate and potassium hydroxide[11-13] and gave the enaminoderivative (IV).

The compound (IV), treated in acetone with ethyl chlorocarbonate, gave the mixed anhydride which was reacted with triethylamonium salt of 6-aminopenicillanic acid and gave 6-(D-α-aminophenylacetamido-1-$^{14}$C)-penicillanic acid trihydrate (V).

## EXPERIMENTAL

Materials and methods.–Melting points are uncorrected, optical rotation were determined at 20° with Perkin-Elmer mod.141.

Infrared spectra were measured in nujol with Perkin-Elmer mod.157 G.

Chromatography:

10 mcg of D,L-α-aminophenylacetic acid-1-$^{14}$C or D-α-aminophenylacetic acid-1-$^{14}$C dissolved in 10 mcl of methanol, 20 mcg of potassium salt of N-(1-ethoxycarbonylpropen-2-yl)-D-α-aminophenylacetic acid-1-$^{14}$C in 10 mcl of HCl N in water, 100 mcg of ampicillin dissolved in 10 mcl of water, were used.

Thin layer chromatography method a: was performed on silica gel treated with buffer pH 6.0 (obtained from 135 ml of 0.1M $Na_2HPO_4$ and 865 ml of 0.1M $KH_2PO_4$) and desiccated 30 min at 105°, using as eluent the superior phase of n-butanol--acetic acid-water 4:1:5 V/V. After the elution and desiccation of the plate, it was sprayed with ninidrin 1% in ethanol and heated at 105° for 10 min.

Thin layer chromatography method b: was performed on Kieselgel GF 254 Merck using as eluent i-propanol-formic acid-water 40:2:10 V/V. After elution and desiccation the plate was sprayed with ninidrin as before.

Thin layer chromatography method c: was obtained on Kieselgel GF 254 Merck using as eluent n-butanol-acetone-triethylamine-water 30:30:6:15 V/V and spraying with ninidrin as indicated before.

Thin layer chromatography method d: was obtained on Kieselgel GF 254 Merck treated with buffer pH 6.0 and desiccated 30 min at 105°, using as eluent i-propanol-formic acid-water 40:2:10 V/V and spraying with ninidrin as indicated before.

Paper chromatography: was performed on Whatman n°4 using as eluent the superior phase of n-butanol-acetic acid-water 4:1:5 V/V. After 6 hours of descending

chromatography, the paper was sprayed with NaOH 0.5N, heated 20 min at 50°, sprayed again with solution obtained from 24.5 g $I_2$, 8 g KI and 200 ml water and mixed with solution obtained from 1 g starch in 100 ml of water and 6 ml of acetic acid: white spots on dark background.

Chemical and biological analyses on the compound (V) were determined according C.F.R. of Food and Drug Administration and reported on anhydrous basis[14], in comparison with an F.D.A. standard.

D,L-α-aminophenylacetic acid-1-$^{14}$C (II).

To a solution of 7.5 g of sodium cyanide (150 mmole) and 1 mCi of Amersham $Na^{14}CN$ in 40 ml of water were added 8.85 g of amonium chloride (165 mmole) and 15.9 g of benzaldehyde (150 mmole) dissolved in 40 ml of methanol. The solution was stirred 2 hours at 20-30°, diluted with 100 ml of water and extracted 2 x 50 ml of benzene. The benzene combined phases were washed 2 x 10 ml of water and then extracted 2 x 40 ml of HCl 20% in water. The acid extracts were refluxed 2 hours, diluted with 150 ml of water and evaporated in vacuo at 50° to 90 ml. The solution was treated with coal, filtered and treated with $NH_4OH$ 32% W/V to pH 7. After 20 hours at 20° the solid product was filtered, washed with water, dissolved in 60 ml of N NaOH and 15 ml of methanol. The solution was again treated with coal, filtered and treated at 60° with HCl 20% in water to pH 6; after cooling at 20°, the D,L-α-aminophenylacetic acid-1-$^{14}$C (II) was filtered, washed with water, dried at 50° (6.9 g), mp 265-8°, IR bands at 1610, 1550, 1510, 1200, 1130, 1070, 1030, 920, 900, 850, 750, 730, 690 $cm^{-1}$.

Only one spot in thin layer chromatography (method a Rf 0.30, method b Rf 0.75, method c Rf 0.0, method d Rf 0.70). The product had SA 0.0364 μCi/mg in HCl N (a sample after 2 crystallizations from ethanol-water 1:1 to constant specific activity gave SA 0.0369 μCi/mg).

Chemical yield 30%, radiochemical yield 25.1%.

D-α-aminophenylacetic acid-1-$^{14}$C (III).

D,L-α-aminophenylacetic acid-1-$^{14}$C (II) (6.8 g, 45 mmole) in 30 ml of water was treated with 11.25 g of D-camphorsulfonic acid monohydrate (45 mmole) at 90° with stirring. The hot solution was filtered, cooled at 0° and the solid product was filtered, washed with 10 ml of water and dried giving 5 g of D-camphorsulfonate of D-α-aminophenylacetic acid-1-$^{14}$C, $[\alpha]_D$ -44° 2% in water. The

mother liquor was treated with NaOH 30% in water to pH 7 at 40°, the solid product was filtered, washed with water, dried giving 4.5 g of product which was refluxed 7 hours with 40 ml of NaOH 10% in water, treated with HCl 20% in water to pH 7, cooled at 0° and filtered giving 4 g of D,L-α-aminophenylacetic acid-1-$^{14}$C (II). From these 4 g of (II), by treatment with D-camphorsulfonic acid as indicated above, 3 g of D-camphorsulfonate of D-α-aminophenylacetic acid-1-$^{14}$C were recovered, $[\alpha]_D$ -43° 2% in water. D-camphorsulfonate of D-α-aminophenylacetic acid-1-$^{14}$C(8 g) was dissolved in 30 ml of water at 80° and treated with NaOH 30% in water to pH 7. After 2 hours at 0° the solid product was filtered and dried *in vacuo* giving 2.9 g of (III), mp 247-51°, IR bands at 1610, 1550, 1510, 1200, 1130, 1070, 1030, 920, 900, 850, 750, 730, 690 cm$^{-1}$, $[\alpha]_D$ -148° 7% in HCl N/2.

Only one spot in the thin layer chromatography (method a Rf 0.35, method b Rf 0.80, method c Rf 0.0, method d Rf 0.70). The product had SA 0.0364 μCi/mg in HCl N (a sample after 3 crystallizations from ethanol-water 1:1 to constant specific activity gave SA 0.0353 μCi/mg).

Chemical yield 42.5%, radiochemical yield 42.5%.

Potassium salt of N-(1-ethoxycarbonylpropen-2-yl)-D-α-aminophenylacetic acid-1-$^{14}$C (IV).

To a solution of 1.27 g of KOH 85% (19.3 mmole) in 20 ml of methanol, 2.83 g of D-α-aminophenylacetic acid-1-$^{14}$C (III) (18.7 mmole) were added; the mixture was treated at 60° with 2.68 g of ethyl acetoacetate (20.6 mmole) dissolved in 12 ml of methanol. The solution was refluxed 20 min, evaporated *in vacuo* to dryness and diluted with 40 ml of isopropanol at 20°. The solid product was filtered, washed with 10 ml of isopropanol and dried *in vacuo* giving 4.25 g of (IV), mp 214-6°, IR bands at 3300, 1650, 1610, 1570, 1270, 1170, 1130, 1070, 1060, 1030, 1010, 950, 940, 780, 730, 700, cm$^{-1}$, $[\alpha]_D$ + 121° 5% in water.

Only one spot in the thin layer chromatography (method a Rf 0.40, method b Rf 0.90, method c Rf 0.0, method d Rf 0.75). The product had SA 0.017 μCi/mg in water (a sample after 2 crystallizations from methanol-i-propanol 1:10 to constant specific activity gave SA 0.0175 μCi/mg).

Chemical yield 75%, radiochemical yield 70.1%

6-(D-α-aminophenylacetamido-1-$^{14}$C)-penicillanic acid trihydrate (V).

To 30 ml of acetone were added, at -40°, 4.2 g of (IV)(14 mmole) and then 1.63 g of ethyl chlorocarbonate (15 mmole), after 15 min at -40° 0.02 ml of N-methylmorpholine were added. The suspension was stirred at -40° for 30 min, and treated at -40° with a solution obtained from 3.24 g of 6-aminopenicillanic acid (15 mmole), 6.6 ml of water, 2.1 ml of triethylamine and 8.6 ml of acetone and previously cooled at -20°. The mixture was stirred 70 min at -40°, filtered, washed with 20 ml of acetone. The filtered solution was evaporated in vacuo at 20° to 20 ml, treated at 0° for 90 min with HCl 30% in water to pH 1, extracted 2 x 10 ml of chloroform. The aqueous phase was treated at 0° with NaOH 30% in water for 90 min to pH 4.5. After a night at 0°, the solid product was filtered, washed with water at 0°, dried at 40° in vacuo, giving 3.4 g of(V),humidity (Karl Fisher) 13.1%, $[\alpha]_D$ + 296° 0.1% in water, iodometric assay 99% of the standard, bioassay 100% of the standard, amine titration 98% of the standard, acid titration 102% of the standard, IR bands at 3400, 1780, 1690, 1610, 1590, 1500, 1330, 1300, 1260, 1220, 1180, 1110, 700 $cm^{-1}$.

Only one spot in the thin layer chromatography with the same Rf as ampicillin standard(method a Rf 0.40, method b Rf 0.90, method c Rf 0.1, method d Rf 0.85) and only one spot in the paper chromatography with Rf 0.50 as the standard. SA 0.0121 μCi/mg in HCl N(a sample after 3 crystallizations from water gave SA 0.0125 μCi/mg).

Chemical yield 60%, radiochemical yield 57.6%.

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