SYNTHESIS OF DISODIUM D(−)−6−(2−PHENYL−2−SULFOACETAMIDO−1−^{14}C) PENICILLANATE (SULFOCILLIN−^{14}C) AND 6−(2−ISOBUTYL−β,γ−$^{3}H_2$−SULFO−2−PHENYLACETAMIDO−1−^{14}C) PENICILLANIC ACID (SP−421−^{3}H, ^{14}C)

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SUMMARY

The syntheses of disodium 6-(2-phenyl-2-sulfoacetamido-1-^{14}C) penicillanate (IV) and 6-(2-isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetamido-1-^{14}C) penicillanic acid (XII) are described. The optically active D(-)-IV was obtained by the fractional crystallization of diastereoisomeric mixture which was prepared from phenylacetic acid-1-^{14}C (I). The compound double labelled with ^{3}H and ^{14}C, XII, was synthesized by the reaction of trimethylsilyl 6-aminopenicillanate with 2-isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetyl chloride-1-^{14}C (XI) which was prepared from isobutanol-2,3-$^{3}H_2$ and I. The overall radiochemical yields of XII, at the specific activities of 9 mCi (^{3}H)/mmole and 2 mCi (^{14}C)/mmole, were 34% yield based on isobutanol-2,3-$^{3}H_2$ and 17% yield based on I respectively.

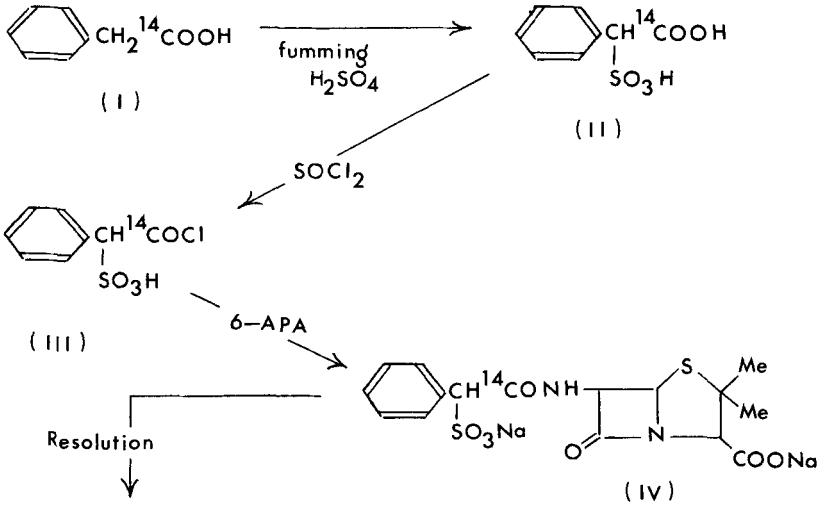
Disodium D(-)-6-(2-phenyl-2-sulfoacetamido) penicillanate (sulfocillin, V),[1,2] a new semi-synthetic penicillin, has a broad antibiotic spectrum against Gram-positive and Gram-negative bacteria. It has been reported in recent years[3-6] that V was especially effective against Pseudomonas and indol-positive Proteus species, and more stable in an aqueous solution than carbenicillin. Animal

studies for acute and subacute toxicities have indicated that V is relatively low-toxic to rodents by any route of administration.[7,8] For studies of the detail metabolic fate in animals, compounds labelled with tritium and carbon-14 were desired. This paper deals with the synthesis of disodium D(-)-6-(2-phenyl-2-sulfoacetamido-1-^{14}C) penicillanate (V-^{14}C) and 6-(2-isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetamido-1-^{14}C) penicillanic acid (XII).

2-Phenyl-2-sulfoacetic acid-1-^{14}C (II) was prepared by the sulfonation of phenylacetic acid-1-^{14}C (I) with fumming sulfuric acid. It was difficult to purify the free acid II due to its hygroscopicity, so that II was converted to disodium salt in 83% yield from I. After purification of the disodium salt, it was reconverted to free acid II by a column chromatography of CG-120

Chart 1

Synthetic route of disodium D(-)-6-(2-phenyl-2-sulfo-acetamido-1-^{14}C) penicillanate



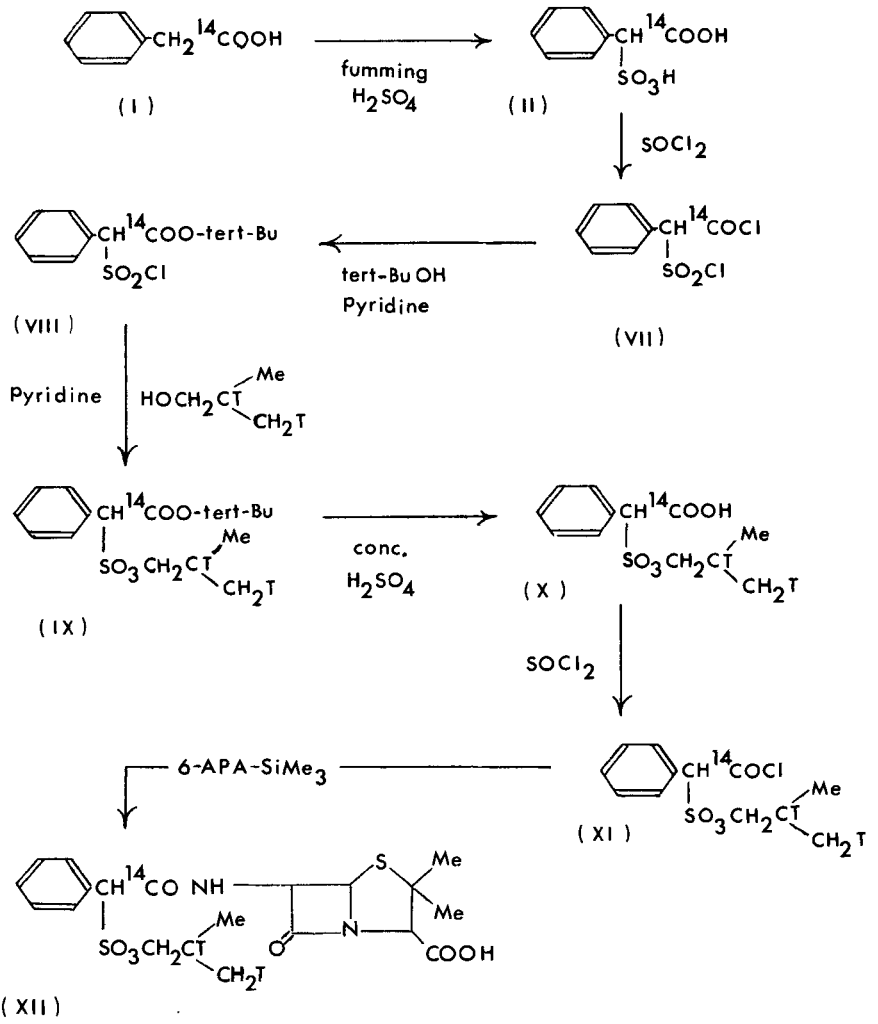
Disodium D(—)-6-(2-Phenyl-2-sulfo-acetamido-1 ^{14}C) Penicillanate (V ^{14}C).

resine. II contains two acidic groups which can be converted into two mono and one di-acid chloride, therefore, the selective preparation of 2-phenyl-2-sulfoacetyl chloride-1-^{14}C (III) was successfully achieved under mild conditions by treating II with thionyl chloride in dried ether containing dimethyl formamide. Schotten-Baumann reaction of 6-aminopenicillanic acid (6-APA) with III in an aqueous solution in the presence of $NaHCO_3$ provided disodium 6-(2-phenyl-2-sulfoacetamido-1-^{14}C) penicillanate (IV), which was purified by passing a column of XAD-2 resine. The diastereoisomeric mixture of IV was separated into two isomers, disodium L(+) and D(-)-6-(2-phenyl-2-sulfoacetamido-1-^{14}C) penicillanate (V-^{14}C), by the fractional crystallization. The overall radiochemical yield from I to V-^{14}C was 9.3%.

The double labelled compound XII was synthesized as shown in Chart 2. 2-Chlorosulfonyl-2-phenylacetyl chloride-1-^{14}C (VII) was obtained by the prolonged reaction of II with thionyl chloride for 192 h at 20°. Synthesis of tert-butyl 2-chlorosulfonyl-2-phenylacetate-1-^{14}C (VIII) by the selective esterification was carried out at -15° to 0° in the presence of pyridine and equimoles of tert-butanol vs. VII, and VIII was further esterified with isobutanol-2,3-$^{3}H_2$ at 0° to 20° in the presence of pyridine to give tert-butyl 2-isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetate-1-^{14}C (IX). The cleavage of tert-butyl ester in IX was effected by mixing with conc. H_2SO_4 to afford 2-isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetic acid-1-^{14}C (X) in 18.5% yield based on I. After the chlorination of X, 2-isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetyl chloride-1-^{14}C (XI) was condensed with trimethylsilyl 6-aminopenicillanate (6-APA-$SiMe_3$) to provide XII. The overall radiochemical yields of XII, at the specific activities of 9 mCi (^{3}H)/mmole and 2 mCi (^{14}C)/mmole, were respectively 34% based on isobutanol-2,3-$^{3}H_2$ and 17% based on I. The purity of XII was 98% based on both radiochromatography and isotope dilution method.

Chart 2

Synthesis of 6-(2-isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenyl-acetamido-1-^{14}C) penicillanic acid



EXPERIMENTAL

Materials

Phenylacetic acid-1-^{14}C and isobutanol-2,3-$^{3}H_2$ were purchased from The Radiochemical Centre, Amersham, England.

2-Phenyl-2-sulfoacetic acid-1-^{14}C (II)

To a mixture of 1 ml of 1,2-dichloroethane, 0.2 ml of fumming H_2SO_4 (containing 50% SO_3) and 0.6 ml of dioxane, 20 mCi of phenylacetic acid-1-^{14}C (2 mmole) in 1 ml of 1,2-dichloroethane was added slowly with stirring at 0°. The resulting mixture was warmed to room temperature, stirred for 4 h, and then allowed to stand overnight at room temperature. To the mixture, 3 ml of water was added with stirring. After the aqueous layer was separated and washed with 3 ml of 1,2-dichloroethane, the pH of aqueous solution was adjusted to 9 to 10 by the addition of 2N NaOH. Then the solution was evaporated under reduced pressure at 55° to give a residue. The residue was dissolved in 6 ml of 80% ethanol, and an insoluble precipitate was removed by filtration. The filtrate was purified with charcoal, diluted with ethanol and allowed to stand in a refrigerator. A fine crystal was collected and dried to provide 523 mg (in 83% yield) of disodium 2-phenyl-2-sulfoacetate-1-^{14}C. An aqueous solution of 523 mg of disodium 2-phenyl-2-sulfoacetate-1-^{14}C was passed over a column of CG-120 resine (30 ml), and then eluted with 80 ml of water. Evaporation of the eluted solution left 324 mg of II (in 75% yield based on I).

Disodium 6-(2-phenyl-2-sulfoacetamido-1-^{14}C) penicillanate (IV)

Into a mixture of 4 ml of anhydrous ether, 1.1 ml of thionyl chloride and one to two drops of dimethyl formamide, 324 mg of II was dissolved and stirring for 50 h at room temperature. To the solution was added 10 ml of petroleum ether and allowing to stand overnight in a refrigerator. An oil layer was separated by decantation and kept overnight at -70° to give 2-phenyl-2-sulfoacetyl chloride-1-^{14}C (III) as a colourless crystal. The crystal of III was washed with 2 ml of petroleum ether and dried under vacuum at 0°. To a mixture of 351 mg of 6-APA in 3 ml of 0.5N NaOH, 1.2 ml of ether and 252 mg of $NaHCO_3$, III in 1.5 ml of ether was added

slowly with stirring at 0° in an ice bath. The resulting mixture was allowed to react at 0° for 30 min, and then the aqueous layer was separated. The aqueous layer was chromatographed on a column of XAD-2 resine (150 ml) with water as the eluent. The eluted fraction of IV was collected and freeze dried in vacuo to provide 366 mg of IV (in 54% yield based on II, specific activity 9.8 mCi/mmole) as a colourless powder.

Resolution of IV

A mixture of 360 mg of IV and 700 mg of unlabelled disodium D(-)-6-(2-phenyl-2-sulfoacetamido) penicillanate was dissolved in 2.2 ml of water, and then the solution was diluted with 11 ml of ethanol and 5 ml of propanol. Fractional crystallization was facilitated by inoculating the solution with a crystal of the species of optically active unlabelled sample. Recrystallization from above solution afforded 428 mg of disodium D(-)-6-(2-phenyl-2-sulfoacetamido-1-^{14}C) penicillanate (V-^{14}C), mp 243-4°, which was identical in every respect with the authentic sample. The overall radiochemical yield and the specific activity of V-^{14}C were 9.3% and 2 mCi/mmole, respectively.

2-Chlorosulfonyl-2-phenylacetyl chloride-1-^{14}C (VII)

A solution of 20 ml of ether, 13.1 ml of thionyl chloride, 0.12 ml of dimethyl formamide was added to 3913 mg of II which had been prepared from 40 mCi, 20 mmole of phenylacetic acid-1-^{14}C (I), and the mixture was allowed to stand for 192 h at room temperature with occasional shaking. The residue was obtained by evaporating the reaction mixture under reduced pressure without heating and extracted with hexane. The extract was evaporated to dryness in vacuo, and the viscous residue was crystallized with cooling at -30° in a dryice acetone bath to give 3173 mg of VII in 63% yield based on I.

tert-Butyl 2-isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetate-1-^{14}C (IX)

A mixture of 1.06 ml of tert-butanol and 1.01 ml of pyridine was added dropwise to a solution of 3173 mg of VII in 5.5 ml of dichloromethane with cooling at -15°, and allowing to stand for 30 min at 0°. A solution of 90 mCi, 10 mmole (745 mg) of isobutanol-2,3-$^{3}H_2$ in 0.81 ml of pyridine was added to the reaction mixture with ice cooling, and then the mixture was stirred for 1 h at 20°. The resulting solution was washed with ice water, 5% HCl, then water, and dried over anhydrous Na_2SO_4. Evaporation of the solution left 2390 mg of IX, 36% yield based on I.

2-Isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetic acid-1-^{14}C (X)

To a solution of 2390 mg of IX in 1.65 ml of dichloromethane, 0.26 ml of conc. H_2SO_4 was added dropwise with ice cooling. The reaction mixture was allowed to stand for 50 min at 0° to -15° and extracted with ether. The ether extract was washed twice with water, and then organic layer was reextracted with 5% $NaHCO_3$ solution. The alkaline solution was made acidic with 5% HCl and the solution was extracted with ethyl acetate. The ester extract was decolorized with charcoal and evaporated to dryness. The residue was dissolved in 1.5 ml of dichloromethane and crystallized by adding hexane. The crystals were filtered off and dried to give 1008 mg of X in 18.5% yield based on I (36.8% yield based on isobutanol-2,3-$^{3}H_2$). Infrared spectrum (KBr) cm^{-1}; 1710 (-COOH), 1365, 1170 ($-SO_2O-$). NMR ($CDCl_3$) δ ppm; 0.96 (d, 6H), 1.60-2.25 (m, 1H), 3.90 (d, 2H), 5.24 (s, 1H), 7.30-7.70 (bs, 5H).

2-Isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetyl chloride-1-^{14}C (XI)

To a solution of 1008 mg of X in 5.4 ml of ether was added 0.35 ml of thionyl chloride and 0.015 ml of dimethyl formamide. The reaction mixture was refluxed for 5 h and then evaporated to dryness. The residue was kept in a refrigerator to affored XI as crystal.

6-(2-Isobutyl-β,γ-$^{3}H_2$-sulfo-2-phenylacetamido-1-^{14}C) penicillanic acid (XII)

To 800 mg 6-APA suspended in 2.2 ml of chloroform was added 1.1 ml of hexamethyl disilazane. The mixture was refluxed for 2.5 h with stirring and evaporated to dryness, and then the residue was dissolved in a mixture of 28 ml of dichloromethane and 0.49 ml of quinoline. The solution was added to a solution of XI in 22 ml of dichloromethane, and the resulting mixture was stirred for 1 h at 0°. To the reaction mixture was added subsequently 76 ml of isopropyl ether, 28 ml of 0.1% HCl and then 28 ml of isopropyl ether with stirring. After the aqueous layer was removed, the organic layer was washed with dil. HCl and water, and dried over anhydrous Na_2SO_4. The dried solution was treated with charcoal and concentrated to 50 ml. The product was crystallized by the addition of hexane, and washed with hexane. Recrystallization from a mixture of dichloromethane and isopropyl ether afforded XII in 17.2% yield based on I and 34.3% yield based on isobutanol-2,3-$^{3}H_2$. The specific activities of XII were 9 mCi (^{3}H)/mmole and 2 mCi (^{14}C)/mmole respectively. The radiochemical purity by isotope dilution method and radiochromatogram was shown to be 98.2%. Infrared spectrum (KBr) cm^{-1}; 1790 (β-lactam), 1760 (-COOH), 1690 (-CONH), 1360, 1170 ($-SO_2O-$). NMR (d_6-DMSO) δ ppm; 0.72-0.82 (d, 6H), 1.30-1.40-1.52 (t, 6H), 1.80 (m, 1H), 3.82 (d, 2H), 4.10 (s, 1H), 5.35 (m, 2H), 5.65 (d, 1H), 7.30 (bs, 5H).

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REFERENCES

1) Morimoto, S., Nomura, H., Fugono, T., Maeda, K. and Ishiguro, T. - Belg. Pat., 739441 (1970).

2) Morimoto, S., Nomura, H., Ishiguro, T., Fugono, T. and Maeda, K. - J. Med. Chem., 15, 1105 (1972).

3) Morimoto, S., Nomura, H., Fugono, T., Azuma, T., Minami, I., Hori, M. and Masuda, T. - J. Med. Chem., 15, 1108 (1972).

4) Akagi, Y., Namikawa, R., Yashiki, T., Matsuzawa, E. and Morimoto, S. - J. Takeda Res. Lab., 30, 453 (1971).

5) Nakazawa, S., Wazima, T., Izawa, T., Tsuda, M. and Hara, Y. - Chemotherapy, 19, 867 (1971).

6) Tsuchiya, K., Oishi, T., Iwagishi, C. and Iwai, T. - J. Antibiot., 24, 607 (1971).

7) Kanno, M., Jimpu, T., Mikoda, T., Shimakawa, H., Maeda, M., Fukuda, T. and Nishikawa, K. - J. Takeda Res. Lab., 30, 248 (1971).

8) Murata, Y., Usui, T., Yamaguchi, H., Kanabayashi, T., Ogiwara, S., Tada, M., Aomori, T., Orita, S. and Yokotani, H. - J. Takeda Res. Lab., 30, 262 (1971).