SYNTHESIS OF 3-(4-CARBAMOYLPYRIDINIOMETHYL)-7β-(2-SULFOPHENYLACETAMIDO)-CEPH-3-EM-CARBOXYLATE MONOSODIUM SALT LABELLED AT [^{14}C]CARBAMOYL AND [1-^{14}C]ACETAMIDO GROUPS

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α-Sulfobenzylpenicillin(1) as a new semi-synthetic penicillin having a broad antibiotic spectrum against Gram-positive and negative bacteria was first prepared in these laboratories and has already been supplied to clinical use. Moreover, many semi-synthetic penicillins and cephalosporins have been synthesized in order to seek for high effective antipseudomonal medicament without toxicity. Among these synthetic and biological studies, 3-(4-carbamoylpyridiniomethyl)-7β-(D-2-sulfophenylacetamido)-ceph-3-em-4-carboxylate monosodium salt (SCE-129) appeared to be one of the most potent antipseudomonal compounds ever known in the β-lactam antibiotics.(2) This paper deals with the syntheses of 3-(4-[^{14}C]carbamoylpyridiniomethyl)-7β-(D-2-sulfophenylacetamido)-ceph-3-em-4-carboxylate monosodium salt (VI) and 3-(4-carbamoylpyridiniomethyl)-7β-(DL-2-sulfophenyl-[1-^{14}C]acetamido)-ceph-3-em-4-carboxylate monosodium salt (XII). By the well known procedure,(3) [carboxyl-^{14}C]isonicotinic acid (III) was obtained from the reaction of $^{14}CO_2$ with 4-pyridyl lithium which was prepared from 4-bromopyridine, and treated with $SOCl_2$, followed by ammonia to give [carbonyl-^{14}C]isonicotinamide (V). Condensation of V with 7β-(D-2-

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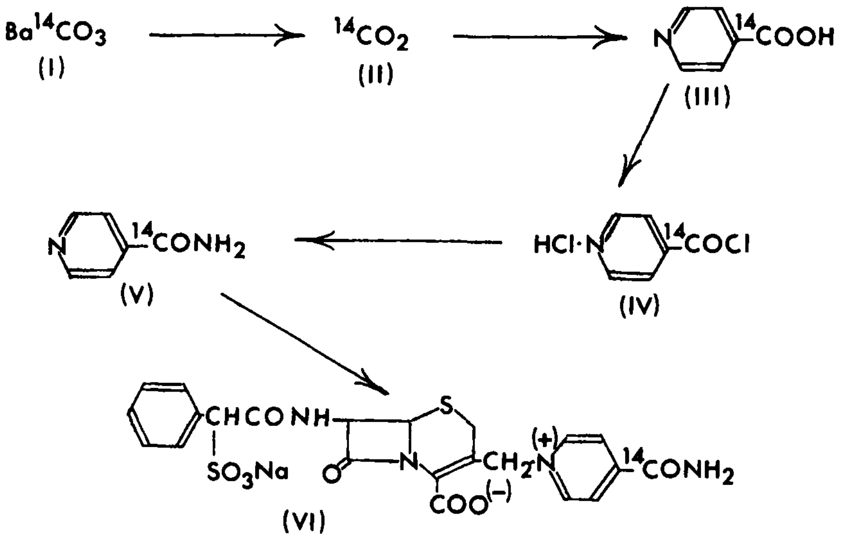
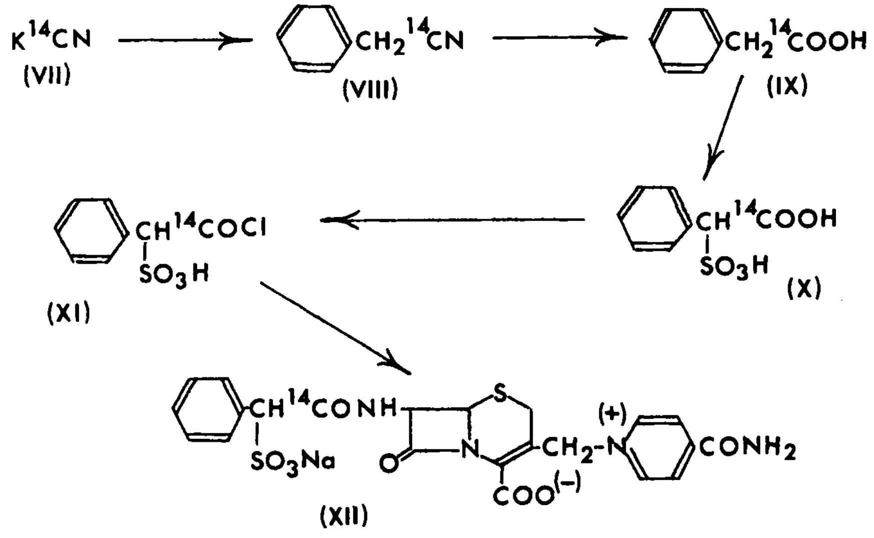
sulfophenylacetamido)cephalosporanic acid disodium salt in the presence of KI gave VI in 27.9 % radiochemical yield based on $Ba^{14}CO_3$.



On the other hand, phenylaceto[^{14}C]nitrile (VIII) was prepared from the reaction of $K^{14}CN$ (VII) with benzylchloride in the presence of benzyltriethylammonium chloride as a phase transfer catalyst,(4,5) and hydrolyzed to phenyl[1-^{14}C]acetic acid (IX) in 87 % yield based on VII. IX was reacted with fuming H_2SO_4, followed by $SOCl_2$ to give 2-sulfophenyl[1-^{14}C]acetyl chloride (XI) in the manner described in the preceding paper.(6) Condensation of XI with



7β-amino-3-(4-carbamoylpyridiniomethyl)-ceph-3-em-4-carboxylate gave XII in 15 % radiochemical yield based on VII.

EXPERIMENTAL

Thin-layer chromatography

Precoated silica gel plates (60 F-254, Merck) were developed in Me_2CO-AcOH-H_2O-28 % aq-NH_3 (36:15:9:2, v/v). Scanning of the thin-layer chromatogram (TLC) was carried out in a TRM-1B scanner (Aloka, Ltd).

High speed liquid-chromatography

A high speed liquid-chromatogram (HLC) equipped with UV detector (254 nm) was used. The column (2.1 mm diameter, 1 m length) was packed with Zipax-SAX (DuPont). Operating conditions: pressure 20 Kg/cm^2, temperature 24° c, chart speed 2.5 mm/min, flow rate of eluent (aqueous solution contining 0.01M NaCl, 0.001M AcONa and 0.001M AcOH) 0.08 ml/min.

[carbonyl-^{14}C]Isonicotinamide (V)

By the well known procedure,(3) [carboxyl-^{14}C]isonicotinic acid was prepared in 73 % yield from 25 mCi (1.6 mmol) of I, and converted to V in 44 % (85.4 mg) yield based on I.

3-(4-[^{14}C]Carbamoylpyridiniomethyl)-7β-(D-2-sulfophenylacetamido)-ceph-3-em-4-carboxylate monosodium salt (VI)

A mixture of 85.4 mg of V, 560 mg of 7β-(D-2-sulfophenylacetamido)cephalosporanic acid disodium salt and 3 g of KI in 1.8 ml of water was heated at 78-70° c for 2.5 h with stirring. After cooling, the KI precipitated was removed by filtration and washed with a few ml of 80 % acetone. Upon cooling in an ice-bath with stirring, the combined solution was added to 70 ml of acetone. The resultant oil separated was caused to crystallize by the addition of 5 ml of acetone with stirring. The crude crystals were washed with acetone, then dried in vacuo and chromatographed over 40 ml of CG-120B (H-form) ion-exchange resin using water as an eluent. The product fractions were checked by thin-layer chromatography (silica gel 60 F-254 (Merck)/Me_2CO-AcOH-H_2O-28% aq-NH_3 (36:15:9:2, v/v)), adjusted to pH 5.5 by adding 0.5N NaOH with cooling and freeze-dried. Recrystallization from a mixture of ethanol and water gave 285 mg of VI containing water of crystallization as colourless needles, Rf. 0.43 on TLC, the retention time of HLC 28.5 min, identical with that of authentic sample. The impure compound in the hydrated crystals of VI was chromatographically indistinguishable from authentic isonicotinamide by both the TLC (Rf. 0.75) and the HLC (retention time, 11 min). No other impure compounds were found. By the HLC, the weight percentages of the anhydrous state of VI and V in the hydrated crystals were determined by comparison with a standard calibration curve and shown to be 91% and 1% respectively. The amount of water of crystallization (VI 2.8 H_2O) was indirectly calculated. The radiochemical purity was 95 % based on both the TLC (Rf. 0.43) and the HLC (retention time, 28.5 min). The specific activity and the radiochemical yield were 14.8 mCi/mmol (24.5 µCi/mg) and 27.9 % based on I respectively.

Phenyl[1-^{14}C]acetic acid (IX)

A mixture of 182 mg (25 mCi/2.8 mmol) of $K^{14}CN$, 443 mg of benzylchloride, 65 mg of benzyltriethylammonium chloride as a phase transfer catalyst(5) and 1.6 ml of water was heated at 98°c for 2 h with stirring. After the addition of 3 ml of water, the aqueous solution was extracted with ether. The ether layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was hydrolyzed with 50 % KOH by refluxing for 3 h. The alkaline solution was extracted with ether in order to remove by-products, then acidified with HCl and extracted with ether. The ether extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. Recrystallization from petroleum ether gave 331 mg of IX in 87% yield based on VII.

3-(4-Carbamoylpyridiniomethyl)-7β-(DL-2-sulfophenyl-[1-^{14}C]acetamido)-ceph-3-em-4-carboxylate monosodium salt (XII)

IX (331 mg) was reacted with fuming H_2SO_4, followed by $SOCl_2$ to give 308 mg of XI in 54% yield based on IX in the manner described in the preceding paper.(6) A solution of 880 mg of 7β-amino-3-(4-carbamoylpyridiniomethyl)-ceph-3-em-4-carboxylate hydrochloride in 1.5 ml of water, maintained below 5° c, was adjusted to pH 6.5 with 2N NaOH. To the solution was added 310 mg of $NaHCO_3$ under cooling in an ice-bath, followed by a solution of 308 mg of XI in 2.6 ml of AcOEt with stirring. To the reaction mixture was added $NaHCO_3$ and the solution was adjusted to pH 5.0. The reaction mixture was stirred for an additional 30 min after which the pH was adjusted to 4.5 with 2N HCl. The aqueous layer was separated, diluted with 30 ml of water and chromatographed over 160 ml of CG-120B (H-form) ion-exchange resin. The product fractions were adjusted to pH 4.8 and freeze-dried. The residue was chromatographically purified with XAD-2 resin. XII (259 mg) with a specific activity of 8.8 mCi/mmol (14.7 μCi/mg) was obtained in 15 % radiochemical yield based on VII. Identity of XII was confirmed by its Rf. 0.43 on the TLC and retention time (DL-form, 28.5 min and 43 min) of the HLC with that of authentic sample. The impure compound in the hydrated crystals of XII was chromatographically indistinguishable from authentic isonicotinamide by the HLC (retention time, 11 min). No other impure compounds were found. By the HLC, the weight percentages of the anhydrous state of XII and isonicotinamide in the hydrated crystals were determined in the manner described in VI and shown to be 92 % and 0.6 % respectively. The amount of water of crystallization (XII 2.5 H_2O) was indirectly calculated. The chemical purity of XII was 97 % based on the HLC.

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