HIGH SPECIFIC ACTIVITY [PHENYL-^{3}H]BENZYLPENICILLIN N-ETHYLPIPERIDINE SALT

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SUMMARY

The N-ethylpiperidine salt of [^{3}H]penicillin G (31 Ci/mmole) was prepared and stored in acetone solution at -15°. The radiochemical purity, as determined by reverse isotope dilution assay, decreased about 12% over a period of one month, less than that expected for an aqueous solution.

Keywords: [phenyl-^{3}H]Benzylpenicillin N-ethylpiperidine salt, non-aqueous [^{3}H]penicillin G solution

High specific activity (HSA) tritiated penicillin G (> 20 Ci/mmole) is potentially useful in biological and pharmacological studies because of the expected high fluorographic and autoradiographic speeds. However, decomposition products resulting from the chemical instability of penicillin G in aqueous solution (1), enhanced by radiochemical decomposition of the tritiated product, may limit the practical utility of the HSA penicillin to only a few days. Although the radiochemical decomposition of HSA compounds may often be minimized by storage at low concentration in alcohol or aqueous alcohol in the cold (2), penicillin G is chemically unstable under these conditions and in protic solvents generally. Therefore, the acetone-soluble N-ethylpiperidine (NEP) salt was prepared (specific activity, > 20 Ci/mmole). Over a period of one month, the NEP salt showed an acceptably small loss in radiochemical purity of 10-12% when stored in acetone solution at -15°. The ease of crystallizing the salt permitted rapid assay of the acetone solution by reverse isotope dilution. The [^{3}H]-benzylpenicillin NEP salt was prepared by the general method of Sheehan and Henery-Logan (3).

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EXPERIMENTAL (7,8)

[Phenyl-^{3}H]phenacyl chloride. Tritiolysis (4,5) of 4-bromophenylacetic acid (100 mg, 0.47 mmole; EtOAc, 2 ml; Et_3N, 0.2 ml; 5% Pd-Al_2O_3, 300 mg; T_2, 1 atm; 10-30 min) and evaporation, acidification, and ether extraction gave tritiated phenylacetic acid (9 Ci; R_f 0.3 (6a); radiochemical purity, 96% (6b)). The dried ether solution (2 ml) was treated with oxalyl chloride (0.10 ml, 1.1 mmole; DMF, 0.002 ml; 30 min, 0-5°) and on evaporation [phenyl-^{3}H]phenacyl chloride was obtained as an oil.

[Phenyl-^{3}H]benzylpenicillinic acid (3). Acetone (30 ml) was added to a solution of D-α-6-aminopenicillanic acid K salt (6-APA, 1.08 g, 5 mmoles; $KHCO_3$, 1.4 g, 5 mmoles; water 35 ml; 0-5°) followed by the above [^{3}H]phenacyl chloride in acetone (3 ml). After 30 min at 0-5° the acetone was evaporated and the aqueous solution was extracted with ether (discarded). The cooled solution was layered with ether (10 ml), acidified to pH 2.6 (50% H_3PO_4), the precipitated excess 6-APA was filtered off, and the aqueous-ether filtrate was extracted with ether. The combined extracts were washed, dried and evaporated, and the residue of [^{3}H]benzylpenicillinic acid was flushed with ethyl acetate (2 ml) to obtain a clear, colorless oil. The product in acetone (3 ml) was treated with a solution of N-ethylpiperidine (0.075 ml, 0.55 mmole) in acetone (0.5 ml) and the NEP salt, which crystallized readily, was collected, washed (acetone, ether), air dried (yield, 102 mg; specific activity, 24.6 Ci/mmole), and dissolved in acetone (0.5 mg/ml; purity, 98.0%; stored at -15°).

Reverse isotope dilution assay (RIDA). The carrier NEP salt was prepared from Na penicillin G (150 mg, 0.42 mmole) by extracting an acidified water solution (4 ml) with ether and treating the benzylpenicillinic acid with N-ethylpiperidine (0.07 ml, 0.51 mmole) as

described in the foregoing procedure (yield, 163 mg (87%); analytically pure). About 50 mg of the NEP salt and the radioactive sample (200 μCi) were dissolved in acetone and the solution was concentrated to 1 ml to obtain the first crop (75-80% recovery). Two recrystallizations were sufficient to obtain a constant specific activity. The results obtained by RIDA of a refrigerated acetone solution of HSA tritiated penicillin G NEP salt (specific activity, 31 Ci/mmole), prepared as described above, are given in the following table.

Radiochemical Stability of [Phenyl-^{3}H] Penicillin G NEP Salt in Acetone (0.5 mg/ml) at -15°

		% by RIDA		
Age, Days	Crop:	1	2	3
1		102.8	102.8	102.5
9		97.3	95.8	95.9
29		91.6	90.2	90.2

REFERENCES AND NOTES

1. See M. Windholz, Ed., "The Merck Index", 9th ed., Merck and Co., Inc., Rahway, New Jersey, 1976, pp. 150-151, for several examples.

2. E. A. Evans, "Tritium and Its Compounds", 2nd ed., Halsted Press, New York, N.Y., 1974, pp. 675-688.

3. J. C. Sheehan and K. R. Henery-Logan, J. Am. Chem. Soc., 84: 2983 (1962).

4. The tritiolysis was performed by the tritium laboratory of New England Nuclear Corp., Boston, MA. The product was flushed with ethanol to remove volatile tritium.

5. D. W. Blackburn and S. H. Levinson in "Catalysis in Organic Syntheses", Academic Press, New York, N.Y., 1976, pp. 82-87.

6. (a) Analtech SG plate developed in toluene-acetic acid 40:1. (b) Determined by thin layer radiochromatographic analysis.

7. Evaporations utilizing a rotary evaporator were carried out below 30°. Magnesium sulfate was used for drying, usually preceded by washing with 30% sodium chloride. Reaction mixtures were magnetically stirred.

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