Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 9

THE SYNTHESIS OF ^{3}H-PUTRESCINE AND SUBSEQUENT BIOSYNTHESIS OF ^{3}H-JACOBINE, A PYRROLIZIDINE ALKALOID FROM SENECIO JACOBAEA

R.L. Reed and D.R. Buhler

Department of Agricultural Chemistry and
Environmental Health Sciences Center
Oregon State University
Corvallis, Oregon 97331, U.S.A.

SUMMARY

A new method was developed for the preparation of tritiated putrescine dihydrochloride ([2,3-^{3}H]-1,4-diaminobutane dihydrochloride) from succinonitrile (1,4-butanedinitrile) and $^{3}H_2O$, with a radiochemical yield of 16%. Tritiated jacobine and other pyrrolizidine alkaloids were then biosynthesized in Senecio jacobaea using ^{3}H-putrescine·2HCl as the precursor with a radiochemical yield of 0.9% into total pyrrolizidine alkaloids. Jacobine accounted for 36% of the total. This synthetic method provides a relatively inexpensive source for the preparation of these labelled compounds.

Key Words: Pyrrolizidine alkaloids, putrescine, Senecio jacobaea, jacobine, tritium.

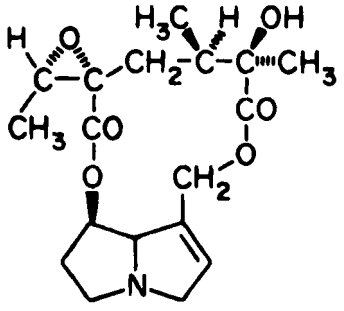
INTRODUCTION

Pyrrolizidine alkaloids (PAs) are hepatotoxic and carcinogenic compounds found in a variety of plants including Senecio jacobaea (1). This plant species is responsible for many cases of livestock poisoning in the western United States (2) and in other areas of the world (3). The major PA present in this species is jacobine [1]. S. jacobaea alkaloids have been shown to be excreted in the milk of cows and goats that have eaten PA-containing plants (4,5) and to also occur in honey (6).

Ingestion of PAs has been implicated as the cause of human veno-occlusive and other diseases in various parts of the world (3,7,8).

0362–4803/88/091041–08$05.00

Received June 15, 1987



JACOBINE

1

Studies on the metabolism and disposition of the PAs have been hampered by the lack of radiolabelled compounds. Chemical syntheses of these alkaloids are difficult because they contain several asymmetric carbon atoms. Use of catalytic tritium exchange is unsuitable because it causes saturation of the necine double bond (1). Several laboratories have prepared radiolabelled PAs through biosynthetic methods. ^{14}C-labelled $NaHCO_3$ has been used in S. vulgaris to produce PAs labelled in all carbon atoms (9). Ornithine has been shown to be incorporated into the PAs of S. jacobaea (10) and several other Senecio species (11-14). Ornithine is selectively incorporated into the necine ring of the PAs (11,15,16). This portion of the molecule binds to cellular electrophiles, resulting in toxicity (17). Putrescine, another molecule selectively incorporated into the necine portion of the PAs, has also been used as a labelled precursor (12,18,19).

These methods are in general plagued by high cost, arising mostly from the expense of the precursors. The present study was undertaken to develop new methods for obtaining relatively inexpensive precursors useful for producing radiolabelled jacobine and other PAs in S. jacobaea for use in animal metabolism studies.

MATERIALS AND METHODS

Chemicals

Succinonitrile and putrescine dihydrochloride were obtained from Sigma Chemical Co., St. Louis, Missouri. Borane-tetrahydrofuran complex (1M in tetrahydrofuran (THF)) and sodium hydride (60% dispersion in mineral oil) were

purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. Absolute ethanol was from U.S. Industrial Chemicals Co., Tuscola, Illinois and HCl gas was from Matheson, Newark, California. Dry THF was freshly distilled over sodium benzophenone ketyl. Deuterated water (99.8%) was purchased from Mallinkrodt Chemical Co., St. Louis and tritiated water (0.225 mCi/mmole) was from Schwarz Mann, Orangeburg, New York. Tritiated putrescine·2HCl (33.1 Ci/mmole; for use as a standard) was obtained from New England Nuclear, Boston, Massachusetts.

Putrescine dihydrochloride

Sodium hydride (0.61 mmoles) was dissolved in 2 ml of dry THF under dry argon and cooled to 0°C. All glassware, etc. was thoroughly dried by vacuum desiccation or oven drying at 130°C. Positive pressure under inert gas was maintained with rubber septa and argon-filled balloons. Succinonitrile (0.67 mmoles, dried in a vacuum desiccator) was dissolved in 2 ml dry THF and added dropwise via a stainless steel cannula to the NaH solution over five minutes with stirring and then allowed to stir a further 15 minutes. Tritiated water (0.56 mmoles, 10 µl, 125 µCi) was added and allowed to react for 5 min. and then nonradioactive water (2 µl, 0.11 mmoles) was added. After 5 min. of further stirring the dinitrile was reduced to the diamine as described in the literature (20,21). Borane-THF complex (8.1 ml of 1 M borane in THF) was added over two minutes and then heated at reflux for 16 hours. Initial attempts at reducing the nitrile with lithium aluminum hydride and with lithium trimethoxyaluminohydride were unsuccessful, possibly due to condensation occurring at acidic hydrogens (22). After cooling to room temperature, 10 ml of absolute ethanol was added and then dry hydrogen chloride was bubbled through the solution for 30 sec. The precipitated putrescine·2HCl was collected by centrifugation and recrystallized from 10 ml 95% ethanol.

Jacobine

First year rosette-stage Senecio jacobaea plants (transplanted from local fields) were injected with an aqueous solution of ^{3}H-putrescine·2HCl into the vascular bundles of their main stems. The 50 µl injections were made via

syringe and needle, and the plants were allowed to take up the liquid at their own rate. After three weeks of growing in a greenhouse the plants were homogenized in a Waring blender and soxhlet extracted overnight with methanol. PAs were extracted from the crude extracts by the method of Culvenor et al. (23). Alkaloids were analyzed and or purified using high pressure liquid chromatography on a Hamilton PRP-1 (Hamilton Co., Reno, Nevada) reversed phase column eluted with acetonitrile (5-25%) and 0.1 M NH_4OH, with UV detection at 220 nm (24).

Analyses

Proton nuclear magnetic resonance spectra (in D_2O) were obtained using a Varian FT-80A spectrometer (Varian Associates, Palo Alto, California) and a Bruker AM400 spectrometer (Bruker Instruments, Billerica, Massachusetts). Infrared spectra were obtained from a Perkin-Elmer 456 spectrophotometer (Perkin-Elmer, Norwalk, Connecticut) using KBr pellets. Mass spectra were obtained by direct probe electron impact using a Finnigan Model 4023 spectrometer (Finnigan, San Jose, California). Radiochemical purity of ^3H-putrescine·2HCl was determined by thin layer chromatography (21) on Uniplate Cellulose GF (Analtech, Wilmington, Delaware) developed with isopropanol:NH_4OH (7:3) and visualized with iodine vapor. Radioactivity was measured in a Packard Model 4530 liquid scintillation spectrometer (Packard, Downers Grove, Illinois) using ACS cocktail (Amersham, Arlington Heights, Illinois).

RESULTS AND DISCUSSION

Tritiated putrescine dihydrochloride was synthesized from succinonitrile and tritiated water. First, sodium hydride was used to abstract acidic protons from the alpha carbons of succinonitrile. The resulting carbanions were then quenched with ^3H_2O to form [2,3-^3H]-succinonitrile, which was then reduced with borane to putrescine and precipitated from solution as the dihydrochloride. The overall chemical yield was 72% and the radiochemical yield was 16%. Thin layer chromatography showed the product to have a radiochemical purity of 96%

and a specific activity of 49 μCi/mmole. Identity was confirmed by IR, NMR, and MS in comparison to commercially available unlabelled material. The IR spectra (KBr pellets) of the synthetic and reference compounds were identical, as were the NMR spectra (80 MHz; C_1 & C_4 protons at 3.02 ppm, C_2 & C_3 protons at 1.75 ppm). Electron impact mass spectra were in agreement, with major peaks at m/e 59, 71 and 89. Deuterated putrescine·2HCl was produced by an analogous synthesis using D_2O in an attempt to confirm the site(s) of deuteration. The mass spectrum of the deuterated product showed peaks at m/e 60, 72 and 90, in addition to the peaks at 59, 71 and 89. Proton NMR (400 MHz) showed the C_2,C_3 peak to be smaller than the C_1,C_4 peak in all of four trial syntheses, thus indicating deuteration at carbons two and three. Comparison of integrated peak areas showed 35 ± 6% (mean ± SEM, n=4) of the product to be deuterated. As expected, this figure is about twice that of the radiochemical yield using tritium, since tritiated water nominally has only one tritium atom per molecule and only one hydrogen atom from each water molecule is added to the anion in the reaction.

Tritiated jacobine was made biosynthetically in S. jacobaea using the ^3H-putrescine·2HCl synthesized from ^3H_2O. Individual plants were given 1.4-10 mg amounts of precursor. The radiochemical yield of total alkaloids was 0.88 ± 0.14% (n=7). In this dosage range, the percent incorporation was apparently unrelated to dose. Jacobine was also biosynthesized from commercially available ^3H-putrescine·2HCl (33 Ci/mmole). At a dosage of only 0.4 μg per plant the radiochemical yield of total alkaloids was 0.87 ± 0.16% (n=5). By HPLC analysis, jacobine accounted for 31.2 ± 5.0% (n=5) of the total alkaloid radioactivity. The other major tritiated alkaloids were seneciphylline and senecionine. Since the incorporation of tritium into jacobine was the same for low and high mass dosings of S. jacobaea, it is concluded that one can treat plants with as much as 10 mg putrescine·2HCl per plant without significantly decreasing radiochemical yield. The identity of isolated jacobine was confirmed by comparing its mass spectrum with that of the previously purified reference compound. Tritiated jacobine was purified to greater than 99% purity by preparative HPLC (24).

Earlier work by Rana and Robins using Senecio isatideus (25) has shown that tritium from the C_2 and C_3 positions of putrescine resides on carbons number 2, 6 and 7 of the pyrrolizidine alkaloid retrorsine. Retrorsine and jacobine both contain the necine base retronecine and differ only in the structure of the esterified necic acid. In the present work, alkaline hydrolysis (23) of the ^{3}H-jacobine to the necine base retronecine using barium hydroxide showed that 99% of the incorporated tritium resided in the necine base (retronecine) portion of the molecule. This is in close agreement with previous work showing over 99% in the necine base of jacobine produced from ^{3}H-ornithine in S. jacobaea (10) and over 95% for retrorsine produced from ^{14}C-putrescine in S. isatideus (26).

Tritiated water is commercially available (Amersham Corp.) at specific activities of up to 1500 Ci/gram. At this activity level, 10 μl contains 15 Ci of tritium and would be expected to produce tritiated putrescine and jacobine with sufficiently high specific activities for use in metabolism experiments. Since ^3H_2O is one of the cheapest sources of tritium, this method provides a relatively inexpensive method for the production of tritiated pyrrolizidine alkaloids.

ACKNOWLEDGEMENTS

We are grateful to the National Institutes of Health (Grants No. CA22524 and ES00210) for support of this work, issued as Technical Paper No. 8165, from the Oregon Agricultural Experiment Station. The authors are indebted to Dr. J.D. White and Mr. T. Somers for their excellent technical advice and gift of dry THF.

REFERENCES

1. Mattocks, A.R., Chemistry and Toxicology of Pyrrolizidine Alkaloids, Academic Press, New York, 1986.
2. Johnson, A.E., Am. J. Vet. Res., 43, 718 (1982).

3. Bull, L.B., Culvenor, C.C.J. and Dick, A.T., The Pyrrolizidine Alkaloids, John Wiley and Sons, New York, 1968.

4. Dickenson, J.O., Cooke, M.P., King, R.R. and Mohamed, P.A., J. Am. Vet. Med. Assoc., 169, 1192 (1976).

5. Deinzer, M.L., Arbogast, B.L., Buhler, D.R. and Cheeke, P.R., Anal. Chem., 54, 1811 (1982).

6. Deinzer, M.L., Thompson, P.A., Burgett, D.M. and Isaacson, D.L., Science, 195, 497 (1977).

7. McLean, E.K., Pharmacol. Rev., 22, 429 (1970).

8. Schoental, R., In Searle, C.E. (ed.), Chemical Carcinogens, American Chemical Society, Washington, D.C., 1976, p.626.

9. Segall, H.J., Brown, C.H. and Paige, D.F. (1983) J. Labelled Compd. Radiopharm., 20, 671-689.

10. Reed, R.L., Reed, B.M. and Buhler, D.R., Planta Medica, 472 (1985).

11. Robins, D.J. and Sweeney, J.R., Phytochemistry, 22, 457 (1983).

12. Bottomley, W. and Geissman, T.A., Phytochemistry, 3, 357 (1964).

13. Bale, N.M. and Crout, D.H.G., Phytochemistry, 14, 2617 (1975).

14. Sorensen, G.G. and Spenser, I.D., Can. J. Chem., 60, 643 (1982).

15. Nowacki, E. and Byerrum, R.U., Life Sciences, 5, 157 (1962).

16. Robinson, R., The Structural Relations of Natural Products, Clarendon Press, Oxford, 1955.

17. Buhler, D.R. and Kedzierski, B., in Kocsis, J.J., Jollow, D.J., Witmer, C.M., Nelson, J.O. and Snyder, R. (eds.), Biological Reactive Intermediates III, Plenum, New York, 1986, p.611.

18. Robins, D.J. and Sweeney, J.R., J. Chem. Soc., Chem. Commun., 120 (1979).

19. Luthy, J., Heim, T. and Schlatter, C., Toxicol. Lett., 17, 283 (1983).

20. Brown, H.C. and Rao, B.C.S., J. Am. Chem. Soc., 82, 681 (1960).

21. Robins, D.J. and Sweeney, J.R., J. Chem. Soc., Perkin I, 3083 (1981).

22. Brown, H.C., Weissman, P.M. and Yoon, N.M., J. Am. Chem. Soc., 88, 1458 (1966).

23. Culvenor, C.C.J., Drummond, L.J. and Price, J.R., Aust. J. Chem., 7, 277 (1954).

24. Ramsdell, H.S. and Buhler, D.R., J. Chromatogr., 210, 154 (1981).

25. Rana, J. and Robins, D.J., J. Chem. Soc., Chem. Comm., 1222 (1983).

26. Khan, H.A. and Robins, D.J., J. Chem. Soc., Chem. Comm., 146 (1981).