PREPARATION OF 3,3'-DICHLOROBENZIDINE-(PHENYL-U-^{14}C) DIHYDROCHLORIDE

W. P. Duncan, J. C. Wiley, Jr., and W. C. Perry
Midwest Research Institute, 425 Volker Boulevard, Kansas City,
Missouri 64110

Received June 10, 1976

SUMMARY

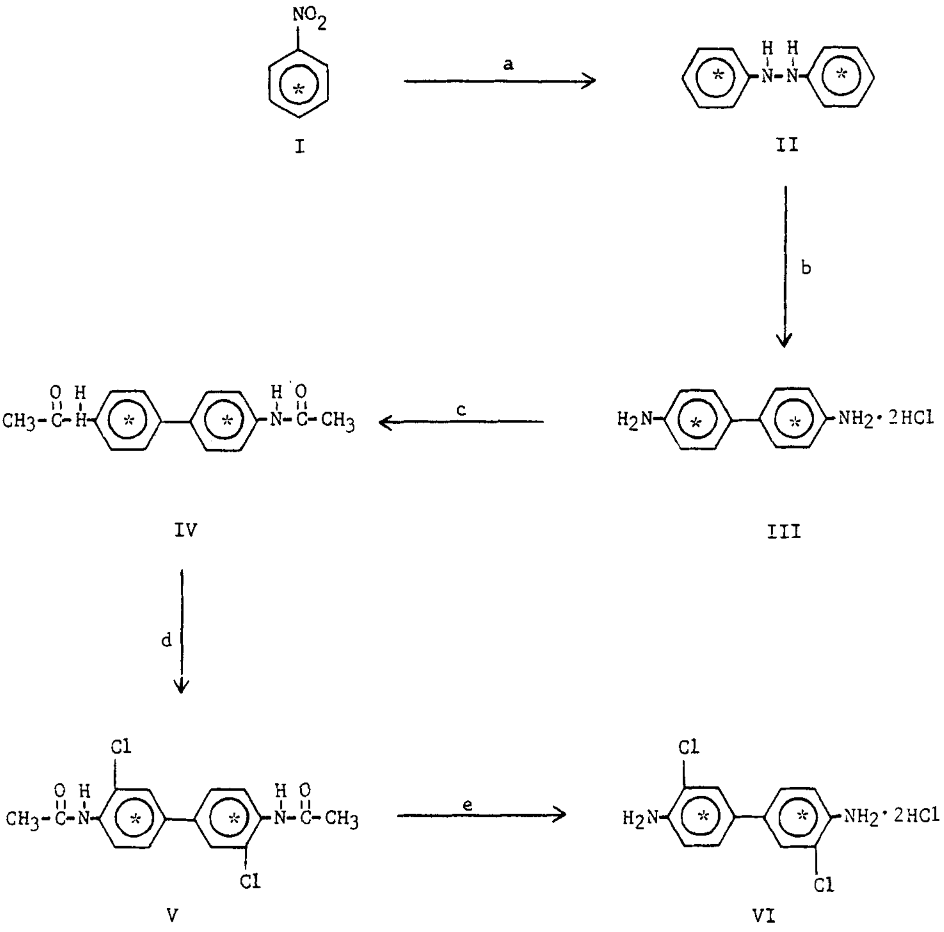
A novel five-step synthesis of 3,3'-dichlorobenzidine-(phenyl-U-^{14}C) dihydrochloride is described. The method consists of the preparation of benzidine-(phenyl-U-^{14}C) followed by acetylation, chlorination with N-chlorosuccinimide (NCS), and hydrolysis. The specific activity of the product was 31.2 mCi/mmole and the overall radiochemical yield was 20% [based on nitrobenzene-(phenyl-U-^{14}C)].

Key Words: 3,3'-Dichlorobenzidine-(phenyl-U-^{14}C) dihydrochloride, Carcinogen, Chlorination, N-Chlorosuccinimide

INTRODUCTION

The widespread use of 3,3'-dichlorobenzidine (DCB) as an intermediate in the manufacture of yellow and orange-red organic pigments [1] and the carcinogenic effects of DCB in experimental animals [2-8] have stimulated interest in the compound for the past several years. This interest is manifested by metabolic studies [9-10] of DCB and by several investigations pertaining to the incidence of cancer in exposed workers [2,11-13]. Further, in May 1973 the United States Department of Labor issued temporary regulations controlling the manufacture and use of DCB [14], and in 1974 the Occupational Safety and Health Administration's list of chemical carcinogens included this material [15].

To facilitate toxicological studies involving DCB, the synthesis of DCB-(phenyl-U-^{14}C) dihydrochloride was required. The method described herein does not necessitate the synthesis of o-nitrochlorobenzene-(phenyl-U-^{14}C) [16] but involves the Ru/C catalyzed reduction of the easily attainable starting material, SCHEME I



^{a}5% Ru/C, NH_2-NH_2, alc. KOH, 40-50°. ^{b}aq. HCl, Et_2O. ^{c}aq. $NaHCO_3$; HOAc, Ac_2O, boil. ^{d}NCS, HOAc, reflux. ^{e}aq. HCl, EtOH.

nitrobenzene-(phenyl-U-^{14}C), see Scheme I. Thus, I was converted to III in 56% overall yield by the acid catalyzed rearrangement of II [17], which in turn had been prepared from I using hydrazine and 5% Ru/C [18]. The free base was then generated from III and acetylated using the procedure of Laham et al. [19] to give IV in 55% yield. Chlorination of IV with N-chlorosuccinimide in glacial acetic acid afforded a 74% yield of V [20] which was subsequently hydrolyzed with hydrochloric acid in ethanol providing the desired compound, VI, in 87% yield [21].

EXPERIMENTAL

Nitrobenzene-(phenyl-U-^{14}C) was obtained from Pathfinder Laboratories, St. Louis, Missouri. Ruthenuim on carbon was purchased from Strem Chemicals, Inc., Danvers, Massachusetts. IR spectra were determined with a Beckman Acculab I, using Nujol. Radioactivity was determined in a Packard Model 3003 liquid scintillation counter using Aquafluor™ (New England Nuclear) as the counting medium. Radiochemical purity was determined using a Packard Model 7201 radiochromatogram scanner. All experimental operations were conducted under a nitrogen atmosphere.

Benzidine-(phenyl-U-^{14}C) dihydrochloride (III)

To 213 μl (2.07 mmole) of nitrobenzene-(phenyl-U-^{14}C) was added 167 μl of 85% hydrazine and 1 ml of 5% alcoholic potassium hydroxide. The stirred reaction mixture was cooled in an ice-bath and 3.0 mg of 5% Ru/C was added. After 1.0 min the ice-bath was removed and the reaction mixture was allowed to warm to room temperature at which time a vigorous exothermic reaction ensued with concomitant precipitation of a gray-white solid. The temperature was maintained at 50-60° by external cooling with an air gun. After stirring an additional 10 min the precipitate was collected by filtration and washed away from the catalyst with 4.1 ml of anhydrous ether. The resulting ether solution of II was suitable for conversion to benzidine dihydrochloride without purification; TLC, R_f 0.45, silica gel/Skelly B:acetone (4:1).

The ether solution of II was cooled in an ice-bath and 4.1 ml of 1:1 hydrochloric acid was added. After stirring 2 min, 2.1 ml of concentrated hydrochloric acid was added followed by rapid stirring for an additional 15 min. The resulting white precipitate was collected by filtration, washed with absolute ethanol (1 x 1 ml), ether (2 x 1 ml), and dried (*in vacuo*) yielding 148 mg (56%, based on I) of III; TLC, R_f 0.56, silica gel/benzene:acetone (1:1).

N,N'-Diacetylbenzidine-(phenyl-U-^{14}C) (IV)

To 148 mg (0.58 mmole) of III dissolved in a minimum amount of warm water was added enough saturated $NaHCO_3$ solution to achieve a pH of approximately 8. The precipitated free base was subsequently collected by filtration and dried (in vacuo). To the dry solid was added 10 ml of glacial acetic acid and 10 drops of acetic anhydride. The resulting suspension was boiled for 30 min, cooled, and the precipitate of crude IV collected by filtration. After recrystallization from ethanol 86 mg (55%) of IV was obtained; TLC, R_f 0.48, silica gel/2-butanone.

N,N'-Diacetyl-3,3'-dichlorobenzidine-(phenyl-U-^{14}C) (V)

To 86 mg (0.31 mmole) of IV suspended in a mixture of 2.6 ml of warm glacial acetic acid and 100 µl of water was added 90 mg (0.67 mmole) of N-chlorosuccinimide in small portions over a period of 15 min. The resulting reaction mixture was slowly heated to reflux. At 100° a completely homogeneous solution was obtained. After approximately 10 min a white solid began to precipitate. The reaction mixture was refluxed for 1 hr, cooled, and the resulting suspension filtered yielding 78 mg (74%) of V as buff needles, m.p. 310-311°, TLC, R_f 0.60, silica gel/2-butanone.

3,3'-Dichlorobenzidine-(phenyl-U-^{14}C) dihydrochloride (VI)

To 78 mg (0.23 mmole) of V was added 30 ml of absolute ethanol and 4 ml of concentrated hydrochloric acid. The resulting suspension was vigorously stirred at reflux for 18 hr, cooled in an ice bath, and the resulting precipitate collected by filtration yielding 65 mg (87%) of VI as an off white crystalline powder, m.p. 275° (decomposition); TLC, R_f 0.60, silica gel/benzene:acetone (2:1). The IR was identical to that obtained on an authentic sample. Radiochromatography indicated a radiochemical purity of $\geq$ 98% [22].

ACKNOWLEDGEMENT

We gratefully acknowledge the support of this work by the National Center for Toxicological Research, Jefferson, Arkansas, BPA No. PIN-428-76.

REFERENCES

1. Lurie A.P. in Encyclopedia of Chemical Technology, 2nd ed., John Wiley and Sons, Inc., New York, New York, Vol. 3, p. 416 (1964).

2. Gadian T. - Chem. and Ind. (London), 821 (1975).

3. Stula E.F., Sherman H., Zapp J.A., Jr. and Clayton J.W., Jr. - Toxicol. Appl. Pharmacol., **31**: 159 (1975).

4. Griswold D.P., Jr., Casey A.E., Weisburger E.K and Weisburger J.H - Cancer Res., **28**: 924 (1968).

5. Sellakumar A.R., Montesano R. and Saffiotti U. - Proc. Amer. Assoc. Canc. Research, **10**: 78 (1969).

6. Pliss G.B. - Voprosy Onkol., **5**: 524 (1959).

7. Pliss G.B. - Khim. Nauka Prom., **4**: 281 (1959).

8. Sabad L.M. - Gig. Tr. Prof. Zabol., **3**: 8 (1960).

9. Sciarini L.J. and Meigs J.W. - Arch. Environ. Health, **2**: 423 (1961).

10. Kellner H.M., Christ O.E. and Lotzch K. - Arch. Toxicol., **31**: 61 (1973).

11. Case R.A.M., Hosker M.E., McDonald D.B. and Pearson J.T. - J. Indust. Med., **11**: 75 (1954).

12. MacIntyre I. - J. Occup. Med., **17**: 23 (1975).

13. Gerarde N.W. and Gerarde D.F. - J. Occup. Med., **16**: 5 (1974).

14. Federal Register, 38(85), May 3, 1973.

15. Chemical and Engineering News, pp. 12-13, February 11, 1974.

16. Normally, DCB is prepared by reduction of *o*-nitrochlorobenzene followed by rearrangement of the intermediate hydrazo compound with hydrochloric acid. This method was deemed untenable for the preparation of the ring-labeled material due to the low radiochemical yield anticipated (30%) for the production of *o*-nitrochlorobenzene by nitration of labeled chlorobenzene.

17. Vogel A.I. Practical Organic Chemistry, Longmans, Green and Company, London, p. 606 (1951).

18. Pietra S. and Res M. - Ann. Chim. (Rome), **48**: 299 (1958).

19. Laham S., Farant, Jean-Pierre and Potvis M. - Ind. Med. Surg., **39**: 142 (1970).

20. These reaction conditions were derived from information reported in the literature describing the direct chlorination of anilines using NCS, Neale et al. - J. Org. Chem., **39**: 3390 (1964); and the chlorination of N,N'-diacetylbenzidine using chlorine gas, Friedländer P. - Fortschritte der Teerfarbenfabrikation and Verivandter Industriezweige, **4**: 73 (1896); and van Roosmalen F.L.W. - Rec. Trav. Chim., **53**: 359 (1934).

21. Whaley T.W - Los Alamos Scientific Laboratory, University of California, unpublished procedures.

22. Numerous "cold" runs in which 1 g of nitrobenzene was used as starting material consistently gave overall yields of 40-45% of unlabeled VI (based on nitrobenzene).