lymphocytic choriomeningitis infection in mice have been reported, and the activity of a third substance, propionin, added. The anti-LCM activities of the 3 natural products have been compared with methotrexate.

The authors are indebted to Dr. H. A. Chirigos, Nat. Inst. of Health, for supplying methotrexate and to Dr. Ralph F. Anderson, International Minerals & Chemical Corp., for supplying crudes containing propionin.

1. Furusawa, E., Cutting, W., Proc. Soc. Exp. Biol. & Med., 1966, v122, 280.

2. Haas, V. H., Stewart, S. H., Virology, 1956, v2, 511.

3. Sidwell, R. W., Dixon, G. J., Sellers, S. M., Schabel, F. M., Applied Microbiology, 1965, v13, 579.

4. Cutting, W., Furst, A., Read, D., Grant, D., Cords, H., Megna, J., Butterworth, E., Antibiot. & Chemother., 1960, v10, 623.

5. Cutting, W., Read, G., Cords, H., Stanford Med. Bull., 1962, v20, 156.

6. Cutting, W., Furusawa, E., 3rd International Congress of Chemotherapy, Stuttgart, 1964, 846.

7. Ramanathan, S., Furusawa, E., Read, G., Cutting, W., Chemotherapia, 1965-66, v10, 197.

8. Ramanathan, S., Read, G., Cutting, W., Proc. Soc. Exp. Biol. & Med., 1966, v132, 271.

9. Rowe, W. P., Black, P. H., Levey, R. H., ibid., 1963, v114, 248.

10. Cook, J. W., Loudon, J. D., The Alkaloids, R. H. F. Manske, H. L. Holmes, ed., Academic Press, New York, 1952, v2, 331.

Received December 22, 1966. P.S.E.B.M., 1967, v125.

2-Amino-1,4-Naphthoquinone, an Oxidation Product of 2-Amino-1-Naphthol.* (32058)

SIDNEY BELMAN, KELVIN FERBER, AND WALTER TROLL
Department of Environmental Medicine, New York University Medical Center New York City And Allied Chemical Corporation Buffalo

The carcinogenicity of aromatic amines is believed to result from their hydroxylated metabolites(1), which are unstable compounds that are easily oxidized. 2-amino-1-naphthol, a metabolite of the bladder carcinogen 2-naphthylamine, produces bladder tumors in about one year when implanted as a wax pellet in bladders of mice(2). Bryan *et al*(3) have shown that within a few hours this compound is largely oxidized in the bladder.

We are studying the interaction of the hydroxylated metabolites with DNA in order to determine their mode of action. The alteration of the melting profile(4) and the ability to prime for RNA synthesis(5) were the induced DNA changes produced by these metabolities. The DNA modifications were obtained only under conditions which allowed oxidation of the metabolites(4); in addition the oxidation products of 2-amino-1-naphthol also altered the melting profile of DNA.

These oxidation products are being examined by us because of the possibility that they are involved in the carcinogenicity of aromatic amines.

Results. The oxidation of 2-amino-1-naphthol (prepared as described in(6) was accomplished by stirring 4 g of the hydrochloride in 2 liters 0.1 M phosphate pH 7.2 for 24 hours at room temperature. The purple precipitate was removed and the yellow-brown supernatant, which exhibited blue-green fluorescence, was extracted several times with ether to give a deep orange extract. The residue obtained after evaporation of the ether layer to dryness was dissolved in methanol and chromatographed on a silica gel thin layer plate in ether/heptane = 2/1 to give 7 bands of various colors. The major orange band was eluted with methanol and evaporated to orange crystals of M.P. = 190-200°. The crystals were extracted with hot benzene

* This investigation was supported by Project Grants from Nat. Inst. Health (USPHS Research Grants CA-09568 and CA-08491) and a grant from Allied Chemical Corp., and is part of a Core Program supported by USPHS, Bureau of State Services Grant ES-00014 and Nat. Cancer Inst. Grant CA-06989.

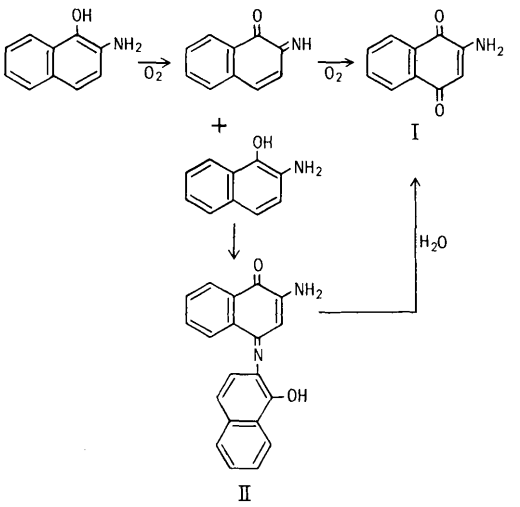

FIG. 1. Oxidation of 2-amino-1-naphthol to 2-amino-1,4-naphthoquinone (1).

and the orange solution separated from a brown oily residue. On cooling, the benzene solution deposited orange needles of M.P. = 213°. The yield was about 50 mg.

The isolated material was identified as 2-amino-1,4-naphthoquinone (I) (Fig. 1) by comparison with an authentic sample prepared according to Fieser(7).

Elemental analysis gave C 69.5, H 4.05, N 8.1, calculated for C_{10} H_7 NO_2; C 69.34, H 3.99, N 7.83.

Recrystallization from n-hexane/benzene gave red needles, M.P. = 210°, authentic compound M.P. = 208°, mixed M.P. = 207°.

The absorption spectra in ethanol and I.R. spectra in KBr of both compounds were identical.

2-amino-1,4-naphthoquinone might be the result of direct oxidation of 2-amino-1-naphthol or of hydrolysis of the dimer (II) (Fig. 1) which is probably the major oxidation product produced(8).

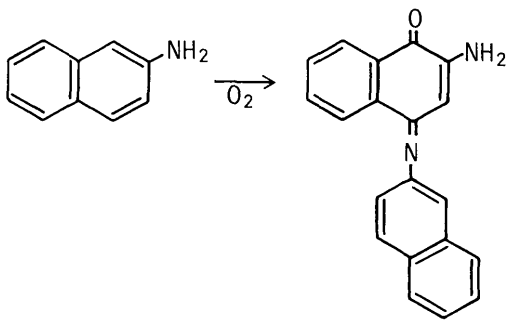

FIG. 2. Oxidation of 2-naphthylamine to 2-amino-1,4-naphthoquinone-N^4-2-naphthylimine.

2-amino-1,4-naphthoquinone was not carcinogenic for rats(9) and did not affect the above mentioned properties of DNA. The aqueous fraction after ether extraction altered the melting profile of DNA and by thin layer chromatography contains at least 10 components. Further studies on this fraction are in progress.

It is of interest that 2-naphthylamine, which is not carcinogenic for mice, produced sarcomas in this species when aged oily solutions were injected subcutaneously. These solutions which developed a red color were recently found to contain the oxidation product 2-amino-1,4-naphthoquinone-N^4-2-naphthylimine (Fig. 2)(10). The carcinogenicity and DNA modifying effects of this compound are not known.

Summary. The carcinogenic metabolite, 2-amino-1-naphthol, of the bladder carcinogen 2-naphthylamine was air oxidized at pH 7.2 to produce many products separable by thin layer chromatography. The ether extract contained one major product which was identified as 2-amino-1,4-naphthoquinone.

1. Clayson, D. B., Chemical Carcinogenesis, Little, Brown & Co., Boston, 1962, p201.

2. Bonser, G. M., Bradshaw, L., Clayson, D. B., Jull, J. W., Brit. J. Cancer, 1956, v10, 539.

3. Bryan, G. T., Brown, R. R., Morris, C. R., Price, J. M., Cancer Res., 1964, v24, 586.

4. Troll, W., Belman, S., Levine, E., ibid., 1963, v23, 841.

5. Belman, S., Huang, T., Levine, E., Troll, W., Biochem. & Biophys. Res. Commun., 1964, v14, 463.

6. Troll, W., Belman, S., Nelson, N., Levitz, M., Twombly, G. H., Proc. Soc. Exp. Biol. & Med, 1959, v100, 75.

7. Fieser, L. F., Experiments in Organic Chemistry, D. C. Heath and Co., Boston, 1957, p234.

8. Nagasawa, H. T., Gutmann, H. R., J. Biol. Chem., 1959, v234, 1593.

9. Belman, S., Troll, W., Teebor, G., Reinhold, R., Fishbein, B., Mukai, F., Proc. Am. Assn. Cancer Res., 1966, v7, 6.

10. Brill, E., Radomski, J. L., Experientia, 1965, v21, 368.

Received December 22, 1966. P.S.E.B.M., 1967, v125.