from the studies of Lee and Soine (5). The acetate (V) as well as the ketone (VI) were available in these laboratories from the studies of Gupta and Soine (28).

Deuteration of lomatin was achieved by heating a small amount of it overnight in a sealed tube at 130° in the presence of enough D_2O to form a clear solution during the heating period. After cooling, the crystallized product was removed by filtration and dried. The ratio of the molecular ion peaks (see Fig. 2) indicated approximately 90% incorporation.

Mass Spectra—These were carried out by Mr. A. R. Swanson and Mr. D. L. Hobbs, School of Chemistry, University of Minnesota, employing a mass spectrometer (Hitachi-Perkin-Elmer RMU-6D). The instrument was operated with a source temperature of 250° and an ionizing voltage of 50 ev. The direct sample inlet temperature was 90° in the case of I, deuterated I, II, V, and VI and 135° in the case of III.

REFERENCES

(1) Willette, R. E., and Soine, T. O., *J. Pharm. Sci.*, **51**, 149(1962).
(2) *Ibid.*, **53**, 275(1964).
(3) Soine, T. O., and Jawad, F. H., *ibid.*, **53**, 990(1964).
(4) Nielsen, B. E., and Soine, T. O., *ibid.*, **56**, 184(1967).
(5) Lee, K. H., and Soine, T. O., *ibid.*, **57**, 865(1968).
(6) Shanbhag, S. N., Mesta, C. K., Maheshwari, M. L., Paknikar, S. K., and Bhattacharyya, S. C., *Tetrahedron*, **20**, 2605(1964).
(7) Seshadri, T. R., Sood, M. S., Handa, K. L., and Vishwapaul, *ibid.*, **23**, 1883(1967).
(8) Shipchandler, M., and Soine, T. O., *J. Pharm. Sci.*, **57**, 741(1968).
(9) *Ibid.*, **57**, 747(1968).
(10) Pirkle, W. H., *J. Am. Chem. Soc.*, **87**, 3022(1965).
(11) Pirkle, W. H., and Dines, M., *ibid.*, **90**, 2318(1968).
(12) Dean, F. M., Goodchild, J., Johnstone, R. A. W., and Millard, B. J., *J. Chem. Soc.*, **1967**, 2232.
(13) Pike, W. T., and McLafferty, F. W., *J. Am. Chem. Soc.*, **89**, 5954(1967).
(14) Bursey, M. M., and Dusold, L. R., *Chem. Commun.*, **1967**, 712.
(15) Brown, P., and Green, M. M., *J. Org. Chem.*, **32**, 1681(1967).
(16) Nakata, H., and Tatematsu, A., *Tetrahedron Letters*, **No. 42**, 4101(1967).
(17) Barnes, C. S., and Occolowitz, J. L., *Australian J. Chem.*, **17**, 975(1964).
(18) Abdel-Hay, F. M., Abu-Mustafa, E. A., El-Tawil, B. A. H., Fayez, M. B. E., Barnes, C. S., and Occolowitz, J. L., *Indian J. Chem.*, **5**, 89(1967).
(19) Abdel-Hay, F. M., Abu-Mustafa, E. A., and Fayez, M. B. E., *Rec. Trav. Chim.*, **86**, 920(1967).
(20) Shapiro, R. H., and Djerassi, C., *J. Org. Chem.*, **30**, 955(1965).
(21) Guise, G. B., Ritchie, E., Senior, R. G., and Taylor, W. C., *Australian J. Chem.*, **20**, 2429(1967).
(22) Aplin, R. T., and Page, C. B., *J. Chem. Soc.*, **1967**, 2593.
(23) Furuya, T., Kojima, H., and Sato, H., *Chem. Pharm. Bull.*, **15**, 1362(1967).
(24) Fisher, J. F., Nordby, H. E., Waiss, A. C., and Stanley, W. L., *Tetrahedron*, **23**, 2523(1967).
(25) Donnelly, B. J., Donnelly, D. M. X., and O'Sullivan, A. M., *ibid.*, **24**, 2617(1968).
(26) Willhalm, B., Thomas, A. F., and Gautschi, F., *ibid.*, **20**, 1185(1964).
(27) Budzikiewicz, H., Djerassi, C., and Williams, D. H., "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, Calif., 1964, p. 254.
(28) Gupta, P. K., and Soine, T. O., unpublished data.

Keyphrases

Coumarins
Lomatin, derivatives—mass spectra
Mass spectra—lomatin, derivatives, fragmentation patterns
Deuterated lomatin—mass spectrum

Radiosynthesis of 3′-Chloro-3-nitrosalicylanilide and Determination of Its Uptake by Lamprey Larvae

By LARRY L. FRENCH and HOWARD A. SWARTZ

Tritium radiosynthesis of 3′-chloro-3-nitrosalicylanilide was accomplished by an exchange reaction with tritiated phosphoric acid-boron trifluoride reagent. Radiochemical purity was established by means of paper chromatography and autoradiography. Residual activity in lamprey larvae was determined at intervals of 1, 2, and 3 hr. The values observed were 3.046, 3.16, and 3.512 percent, respectively, representing a total residue of 0.0182 mg./g., 0.0186 mg./g., and 0.021 mg./g. at the stated time intervals.

THE LAMPREY EEL of the Great Lakes has inflicted considerable damage to the fishing industry of this region, and for many years attempts have been made to kill the eel and inhibit its reproduction.

The lamprey eel is a vertebrate of the Cyclostomi, the "round mouthed" class, constituting the *Petromyzon* genus. Although there are five species indigenous to the Great Lakes region, both

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parasitic and nonparasitic, the species *marinus* was chosen for this work because they are the most abundant and harmful to the industry. Young eels, called ammocoetes, remain in the larval stage some 3 years. Although delectible food for other fish, they spend most of their time buried in the soft mud and sand at the bottom of the lake for protection. It is the larva that is affected by chemical methods of eradication. Partial eradication has been attributed to the widespread treatment of the larval habitat with 3-trifluoromethyl-4-nitrophenol, known commercially as TFM (1). The larvacidal LD_{100} for this compound varies from 3–10 p.p.m., depending on the physical and chemical characteristics of the water in which it is used. The LD_{25} for 11 species of fish indigenous to this area has been reported to be between 5–42 p.p.m. (2). During a continuing screening program to detect more potent and selectively toxic lamprey larvacides, substituted 3-nitro- and 5-nitrosalicylanilides were found to be highly active with relation to TFM. One compound, 2′-5-dichloro-4′-nitrosalicylanilide,[1] has been found and used successfully as a synergist to TFM in the control of the lamprey. Many derivatives of the salicylanilide series have been found to exhibit a diverse bioactivity spectrum, including antimicrobial activity (3), molluscidal activity (4), antitumor activity (5), and as a taenecide (6). Preliminary mammalian oral toxicity studies with substituted 3-nitro- and 5-nitrohalo compounds of this series indicate lack of side effects in dosages less than 2.5 g./kg. of body weight (7). Prompted by the preliminary larvicidal screening studies and relatively low mammalian toxicity, it was decided to evaluate the structural-activity relationships of a series of 3-nitro and 5-nitrosalicylanilides (8). From these studies it was found that the obligatory molecular requirement for activity was the orthophenolic substituent on the carboxylic acid moiety of the salicylanilide. In addition, maximum toxicity and selectivity required the optimum combination of a halogen and a nitro group in the molecule. Substitution of more than two of the halogens in the aniline moiety, however, decreased selectivity and toxicity due to a saturation effect (9).

Studies by Starkey and Howell (10) showed that the combination 3′-chloro-3-nitrosalicylanilide, known experimentally as 33NCS, produced maximum toxicity and selectivity. The LD_{100} for this compound for the larvae was determined to be 0.3 p.p.m. and an LD_{25} for fingerling rainbow trout to be 0.9 p.p.m. The compound was selected for the study reported here.

A primary problem facing the investigator desiring to employ radiotracer methods is securing the suitable labeled compounds. The two major elements of radiolabeling are ^{14}C and ^{3}H. Chemical syntheses employing these two isotopes are used frequently to prepare labeled compounds in many tracer studies. Tritium-labeled compounds have been increasingly used in recent years, even though the mean energy of the β-particles emitted is only 5.5 kev. However, with the advent of highly sensitive scintillation systems, the usefulness of this isotope has been greatly exploited. Tritium-labeled compounds are usually "hotter" in terms of specific activity than other isotopic compounds available for biological investigation. Tritium radiosynthesis may be accomplished by several methods, none offering a simple procedure but having the distinct advantage of the high specific activity at relatively low cost.

The method of choice for most biological tracer studies with tritium is the reduction of a suitable unsaturated precursor with the carrier-free tritium gas, carried out in a nonoxidizing solvent under highly controlled criteria of study and evaluation. The advantages of this system are a very high specific activity, purity of the product, and the known position of labeling of the compound. The major limitation to this method, however, is the availability of the suitable precursor of the desired compound and the criteria for the reaction procedure.

Random tritium labeling may also be secured by simple exchange methods, with or without a catalytic system. Usually this method employs either tritiated water or glacial acetic acid as the exchange medium and platinum black as the catalyst (11). Although high specific activities are available through this method, much of the tritium exchanged product will be labile, and will exchange, or reverse its initial exchange, upon contact with nonlabeled material. The removal of the labile tritium and subsequent purification may be very difficult to achieve.

A third method, the Wilzbach reaction (12), or the Wilzbach gas exposure method, is largely responsible for the increased popularity of tritium as a tracer. In this method, the compound to be labeled is exposed to curie amounts of carrier-free tritium gas in a sealed reaction vessel for a period of from a few days to several weeks. External energy such as electric discharge, microwaves, UV irradiation, and γ irradiation have been used to hasten the process, yielding, however, many

[1] Bayer 73.

more degradation products. The energy released in the disintegration of the tritium and absorbed by the system provides the activation necessary to effect labeling. The major disadvantage of such a system is the formation of a great number of by-products, due largely to radiolysis. Rigid and extensive purification steps are necessary to insure radiochemical purity.

An excellent method for tritium exchange was reported in the literature by Yavorsky and Gorin (13), and has been successfully used to label several compounds (14). The method involves the preparation of an exchange reagent, tritiated phosphoric acid-boron trifluoride ($3H_3PO_4 \cdot BF_3$). Many organic compounds can be labeled with tritium by simply mixing the compound to be labeled with the reagent and agitating this mixture in a sealed vessel for several hours. The ultimate specific activity theoretically can be calculated from the formula (14):

$$Sx = \frac{S_R{}^0}{(3/166)\,(M_X/N_H) + (W_X/W_R)}$$

where $S_R{}^0$, initial specific activity of the reagent; M_X, molecular weight of the product; W_X/W_R, weight ratio of product to reagent; N_H, number of exchangeable hydrogen positions of the compound to be labeled.

Studies with this procedure have shown that compounds having substituent groups that tend to withdraw electrons from the aromatic ring inhibit substitution of the tritium. This includes groups such as the nitro- and carboxyl- substituents. Substituent groups that contribute electrons to the ring, such as alkyl and aryl moieties, tend to activate exchange. This method appeared to offer a suitable approach to labeling the selected compound, 33NCS, with the distinct advantages of low radiation hazard and reduced purification requirements.

EXPERIMENTAL

Preparation of the Labeled Reagent—Tritiated phosphoric acid saturated with boron trifluoride (BF_3) was prepared by the method of Yavorsky and Gorin (13). Two-tenths milliliter of tritiated water, with an initial specific activity of 500 mc./ml., was diluted to 5 ml., using triple-distilled deionized water. This combination was thoroughly mixed and added to 13.1 g. of anhydrous phosphorus pentoxide, in a sealed reaction chamber that had previously been purged with nitrogen. The apparatus employed is illustrated in Fig. 1. When all of the tritiated water had been added to the reaction flask and the dense fumes had dissipated, the mixture was saturated with the boron trifluoride (see Fig. 1).

The stoichiometric equation for the reaction is as follows:

$$6BF_3 + 9\ {}^3H_2O + 3P_2O_5 \rightarrow 6\ {}^3H_3PO_4 \cdot BF_3$$

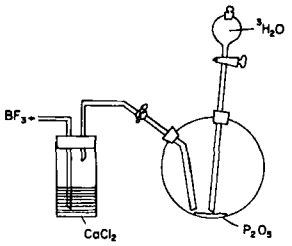


Fig. 1—Apparatus for radiosynthesis.

The final product is a very dense, viscous, yellow liquid.

Preparation of the Tritium-Labeled Compound—To the labeled reagent, 2.000 g. of the compound 33NCS[2] was added. This mixture was then heated to 40° and mixed by means of magnetic stirrers for a period of 24 hr. At the conclusion of this period, the mixture was allowed to reach equilibrium at room temperature and the flask containing the labeled mixture removed from the apparatus and the labeled 33NCS precipitated from solution by the addition of a 50-ml. divided quantity of purified deionized distilled water. The precipitate was collected on filter paper and dried in an oven at 37°. The total weight of the dried, but unpurified, product was 1.818 g.

Radiochemical Purity of the Compound—Several solvent systems were evaluated for recrystallization of the labeled product for purification purposes. The most efficient and convenient of these was acetone. This solvent proved to be superior for two reasons: first, upon solution and recrystallization, excellent yield was obtained, and second, acetone allowed sufficient removal of labile tritium on the labeled compound.

The labeled compound was recrystallized four times from acetone in each of three separate quantities. Samples were taken from each division on each occasion of recrystallization, and it was observed that constant specific activity was obtained after the third recrystallization. After the total recrystallization effort, the combined weight of labeled 33NCS was 1.050 g.

A 20-mg. sample of the purified product was added to 50 ml. of toluene, agitated to obtain complete solution, and allowed to reach equilibrium. Two 0.01-ml. samples of this solution were spotted on separate Whatman No. 1 chromatographic paper. The spots were air dried, the papers placed in a chromatographic chamber, allowed to attain equilibrium. One chromatogram was prepared using a solvent system of butanol 4 parts, water 4 parts, and glacial acetic acid 1 part (No. 1). The other solutions consisted of butanol 4 parts, and water 1 part (No. 2). The individual chromatograms were placed on sheets of 8 × 11 film (No-Screen Kodak X-Ray), and exposed for 10 days. At the end of this period, the films were removed and developed. In both instances, a single, well-defined exposed area was observed, indicating complete radiochemical purity.

Chemical Yields and Final Specific Activity—The final specific activity of the labeled 33NCS was determined by taking 1 ml. of solution, containing 200 mg. of labeled compound in 20 ml. of toluene, and diluting again to 10 ml. in toluene. A 0.1-ml. aliquot was taken for counting in a liquid scintillation detec-

[2] 3′-Chloro-3-nitrosalicylanilide, supplied by Ben Venue Laboratories, Inc., Bedford, Ohio.

tor.[3] These samples were counted at the determined tritium balance point for this instrument.

Each sample was counted under these conditions and the necessary corrections made. The quenching effect was corrected by means of an internal standard of tritiated hexadecane. The true net counts per minute (c.p.m.) for each sample were then calculated and the specific activity in net c.p.m./mg. determined. This value was calculated and found to be 1.32 μc./mg. of labeled compound 33NCS.

Determination of Residual Activity of Lamprey Larvae—A stock solution of the labeled 33NCS was prepared by dissolving 200 mg. in 2 l. of deionized water. Aliquots of 6 ml. of this solution, containing 0.6 mg. of labeled compound, were diluted to 3 l. to give the maximum toxic dose of 0.2 mg./l., or 0.2 p.p.m. The water employed for the 3-l. volumes was aged to afford removal of chlorine. The volumes were aerated and six lamprey larvae[4] added to each of three volumes. Aeration was continued at a constant rate throughout the experiment. One group of the lamprey larvae was removed at the end of the first hour after exposure to the labeled 33NCS. The second group was removed at the end of 2 hr., showing extremely toxic effects of the compound. The final group was removed at the end of the third hour. These larvae were completely immobilized, though not yet dead at the time that they were removed. Each individual larva for each group was accurately weighed (see Table I for individual weights) and placed into individual glass vials containing 25 ml. of a solution of 1 *M* diisobutylcresoxy ethoxyethyl dimethyl benzyl ammonium chloride[5] in methanol. The vials were then sealed by means of aluminum lined caps and incubated at 37° to facilitate digestion and solubilize the larvae. When these processes were complete, 1-ml. samples were taken for counting.

All samples were then counted by means of a liquid scintillation counter.[6] The 1-ml. samples for counting were added to 15 ml. of a liquid scintillation counting solution contained in Wheaton No-Sol-Vite glass vials. This solvent system employed consisted of PPO (2,5-diphenyloxazole), 0.3%; POPOP [1,4-bis-2-(5-diphenyloxazoyl) -benzene], 0.1%; naphthalene, 5%; cellusolve, 300 ml., dioxane, 300 ml.; and toluene, a quantity sufficient to yield 1 l.

The activity of the initial 0.2 p.p.m. solution was determined by taking 1 ml. of the original solution and adding to 15 ml. of the solvent system. The activity of the standards were determined at the determined balance point for tritium. A counting time of 10 min. was employed for all samples. The counting error was calculated to be less than 1% in all instances. Quenching again was corrected by means of an internal standard of tritiated hexadecane.

The percent residue for each larvae at each interval was determined by comparing the residual activity/g. to the total activity of the 3-l. solution. These values are tabulated in Table I and illustrated in Fig. 2.

From the known activity/ml. of initial solution and the total activity per body weight of each larva, the total milliliters of the solution of the compound 33NCS cleared by each eel were calculated, and the values tabulated in Table II.

TABLE I—PERCENT RESIDUE OF LABELED SOLUTION PER GRAM OF LAMPREY LARVAE

No.	Wt., g.	% Residual Activity/g.
	1-hr. Interval	
1	0.6914	2.72
2	1.310	3.32
3	0.3914	3.37
4	0.5202	2.95
5	0.4720	2.88
6	0.7242	3.04
$\bar{X}$	—	3.046
SX	—	0.232
	2-hr. Interval	
1	1.330	2.59
2	1.610	2.69
3	0.4916	4.24
4	0.6712	3.16
5	0.4932	3.19
6	1.061	3.09
$\bar{X}$	—	3.16
SX	—	0.533
	3-hr. Interval	
1	1.4912	2.90
2	0.8394	3.88
3	1.1204	3.40
4	0.7236	3.73
5	0.4974	3.47
6	0.5286	3.69
$\bar{X}$	—	3.512
SX	—	0.319

DISCUSSION

Radiosynthesis—Tritium labeling of compound 33NCS (3′-chloro-3-nitrosalicylanide) was achieved by use of tritiated phosphoric acid saturated with boron trifluoride. This method offers a distinct advantage over the recoil methods in the ease of recovery and purification. Three to four recrystallizations gave a radiochemical pure product, with very little loss of compound. The final specific

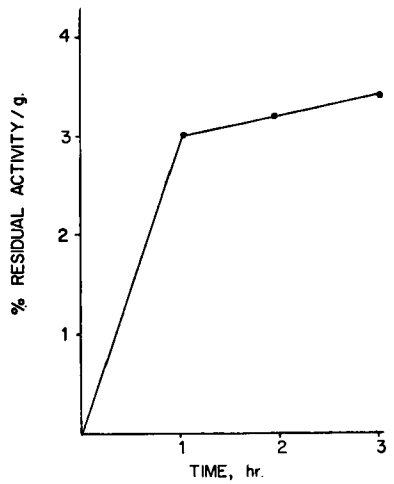


Fig. 2—Percent residual activity versus *time.*

[3] Ecko detector model N610A, Beta spectrometer, model N664A.
[4] The larvae were supplied by Mr. John Howell, Hammond Bay *Biological Station*, United States Department of the Interior, Millersburg, Mich.
[5] Hyamine, Rohm and Haas, Philadelphia, Pa.
[6] Packard Tri-Carb Counter and Detection System, model 314-S.

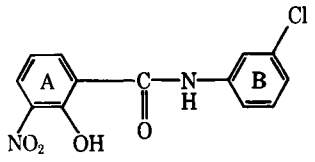
TABLE II—TOTAL MILLILITERS OF COMPOUND 33NCS SOLUTION CLEARED BY EACH LARVA

No.	ml./g.[a]	ml./Body Weight[a]
	1-hr. Interval	
1	81.55	56.38
2	99.60	129.48
3	101.13	39.58
4	88.52	46.05
5	86.29	46.73
6	91.10	65.97
$\bar{X}$	91.36	63.03
SX	6.992	31.101
	2-hr. Interval	
1	75.88	113.17
2	80.81	67.83
3	127.42	142.76
4	94.52	68.39
5	95.81	47.65
6	92.75	49.03
$\bar{X}$	94.53	81.47
SX	16.444	34.915
	3-hr. Interval	
1	87.09	115.83
2	116.54	187.62
3	102.04	50.06
4	112.11	75.24
5	104.04	51.31
6	110.91	117.68
$\bar{X}$	105.76	99.628
SX	9.663	47.813

[a] Calculated from percent residue per gram and body weight and total initial activity.

activity was 1.32 μc./mg. or 1/32 mc./g. which proved satisfactory for the uptake study even at the low concentration of 0.2 p.p.m. (0.2 mg./l.).

The position of the label, as with all tritium exchange methods, was random. The structure of the product, (I) has a bearing on the degree of exchange



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(14). The nitro and carboxyl groups in Ring A would tend to suppress tritium exchange in this ring as they are ring-deactivating groups. This would perhaps be overcome to some extent by the ring-activating hydroxyl group. The chlorine in Ring B would have little influence on exchange. The hydrogen of the A ring hydroxyl and the amino on Ring B would readily exchange, but being labile, would be removed in purification. On the basis of the electrophilic action of the carboxyl and nitro groups, with their inhibition of exchange in Ring A, it is estimated that Ring B contained the predominate number of tritium atoms and the label is principally found in this ring.

Larvae Residual Activity—The mean value in percent for the residual activity/g. were 3.046 ± 0.232 at 1 hr., 3.16 ± 0.533 at 2 hr., and 3.512 ± 0.319 at 3 hr. As illustrated in Fig. 3, these values are indicative of a very rapid uptake from the surrounding medium with a slight but gradual increase from 1 hr. to the 3-hr. period. At this time, all of the larvae were immobilized and showing severe toxic effects. None were dead. The mean values for the milliliters of solution cleared of the labeled compound for the time intervals involved were 91.36 ± 6.992/g. or 63.03 ± 31.101/total body weight, 94.53 ± 16.44/g. or 81.47 ± 34.915/total body weight, and 105.46 ± 96.63/g. or 99.628 ± 47.813/total body weight, respectively. These values do not take into consideration that the actual concentration was being reduced with time, and are therefore somewhat low. Based on these values the total uptake of the labeled compound, calculated from 0.0002 mg./ml. × ml. cleared/g., are 0.0182 mg./g. at 1 hr., 0.0186 mg./g. at 2 hr., and 0.021 mg./g. at 3 hr. Similar, but slightly higher, values are obtained by calculating the total uptake from the percent residues/g. and the initial concentration of the compound (% residue/g./100 × 0.6 mg.). Thus on the values observed, it appears that an uptake of some 0.02 mg./g. of 33NCS produces severe toxicity in the larvae. This could be low also, as the figure is based on the observed residual activity/g. and it must be recognized that metabolic products could have been formed and excreted, which would reduce the residual activity. The milliliters of water cleared/g. at each time interval would not apply for the natural habitat as in this study a uniform or homogeneous distribution was employed which could not be expected in a lake or river.

SUMMARY

Radiosynthesis of the compound 33NCS proved quite satisfactory. The distinct advantages of the method for tritium labeling gave a radiochemically pure product in good radio yields.

The calculated dose of 0.2 p.p.m. appears to be quite toxic to the larvae. Toxic reactions and indications were observed at the end of 1 hr. and by the end of 2 hr., the larvae were having extreme difficulty in maintaining the righting reflex. By the end of 3 hr., all the larvae were completely immobilized and very near death.

REFERENCES

(1) Applegate, V. C., Howell, J. H., Hall, A. E., and Smith, M. A., U. S. Fish and Wildlife Service, Special Scientific Report No. 207, 157(1957).

(2) Applegate, V. C., and King, E. L., Jr., *Trans. Am. Fisheries Soc.*, **91**, 342(1962).

(3) Taborski, R. G., Darker, G. D., and Kaye, S., *J. Pharm. Sci.*, **48**, 503(1959).

(4) Gonnert, R., and Strufe, R., *Ciba Found. Symp.* Little, Brown, Boston, Mass., 1962, p. 326,

(5) Taborsky, R. G., and Starkey, R. J., *J. Pharm. Sci.*, **51**, 1152(1962).

(6) Forbes, L. S., *Vet. Rec.*, **75**, 321(1963).

(7) Taborsky R. G., and Starkey, R. J., *J. Pharm. Sci.*, **52**, 542(1963).

(8) Starkey, R. J., Ben-Vue Laboratories, Inc., Cleveland, Ohio, personal communication, 1965.

(9) Starkey, R. J., and Howell, J. H., "Substituted Nitrosalicylanilides: A New Class of Selectively Toxic Sea Lamprey (*Petromyzon marinus*) Larvacides," U. S. Bureau of Commercial Fisheries, Hammond Bay Biological Station, Millersburg, Mich., 1965, p. 10.

(10) *Ibid.*, p. 12.

(11) Wang, C. H. and Willis, D. L., "Radiotracer Methodology in Biological Science," Prentice Hall, Englewood Cliffs, N.J., 1965, p. 237.

(12) *Ibid.*, p. 238.

(13) Yavorsky, P. M., and Gorin, E., *J. Am. Chem. Soc.*, **84**, 1071(1962).

(14) Yavorsky, P. M., and Gorin, E., *Isotopes and Radiation Technology*, **1**, 752 (1964).

Keyphrases

3′-Chloro-3-nitrosalicylanilide, tritium labeled—synthesis
Paper chromatography—analysis
Radiochromatography—analysis
Liquid scintillation counting—radioactivity
Lamprey larvae—3′-chloro-3′-nitrosalicylanilide effect

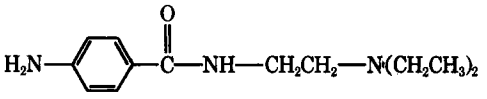
Potential Antiarrhythmic Agents I

Synthesis and Pharmacological Evaluation of Some Piperazine and Ethylenediamine Analogs of Procaine Amide

By D. K. YUNG*, L. G. CHATTEN, and D. P. MACLEOD*

Fourteen 1-alkyl-4-benzoyl-, 1-alkyl-4-*p*-substituted benzoylpiperazine hydrochlorides and eight *N,N′*-dibenzoyl-, *N,N′*-di(*p*-substituted benzoyl)-*N*-alkylethylenediamines were synthesized as procaine amide analogs. These compounds were tested for their effect on the effective refractory period of isolated guinea pig atria. 1-*n*-Propyl-4-benzoylpiperazine hydrochloride, 1-*n*-butyl-4-benzoylpiperazine hydrochloride, 1-*n*-propyl-4-*p*-methoxybenzoylpiperazine hydrochloride, 1-*n*-propyl-4-*p*-chlorobenzoylpiperazine hydrochloride, 1-isobutyl-4-*p*-chlorobenzoylpiperazine hydrochloride, 1-*n*-pentyl-4-*p*-chlorobenzoylpiperazine hydrochloride, and the eight *N,N′*-dibenzoyl-, *N,N′*-di(*p*-substituted benzoyl)-*N*-alkylethylenediamines were further screened for antiarrhythmic activity in cats. Only 1-*n*-propyl-4-*p*-chlorobenzoylpiperazine hydrochloride showed some degree of protection against aconitine-induced cardiac arrhythmias.

THE RELATIONSHIP between the structure and the antiarrhythmic activity of procaine amide [*N*-(2-diethylaminoethyl)-*p*-aminobenzamide, I] has not been thoroughly studied. A few



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workers, however, have studied the alteration of antiarrhythmic activity caused by various substituents on the benzene ring. Clinton *et al.* (1) reported that several of the higher members of the *N*-(2-diethylaminoethyl)-4-amino-2-alkoxybenzamides, in particular the 2-hexoxy derivative, showed outstanding properties as antiarrhythmic agents. Reisner and Cordasco (2) synthesized 2-chloroprocaine amide and found that this compound was approximately four times as potent as procaine amide in blocking atrial fibrillation in dogs. Libonati and Segre (3) studied the antiarrhythmic activity of *N*-(2-diethylaminoethyl)-*p*-4-hydroxybutylbenzamide and *N*-(2-diethylaminoethyl)-*p*-isopropylbenzamide and reported that these compounds showed an activity of 75 and 10 times, respectively, that of procaine amide. Similar compounds, such as *N*-(2-diethylaminoethyl)benzamide and *N*-(2-diethylaminoethyl)-*p*-methoxybenzamide, have also been evaluated for antiarrhythmic activity (4). The former compound was found to have a lower toxicity but the same potency as procaine amide and the latter compound to have a higher potency and toxicity than procaine amide.

The first systematic synthesis of procaine amide analogs was reported by Thyrum and Day (5). In the series of compounds they prepared, the following showed significant antiarrhythmic activity when tested on isolated

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