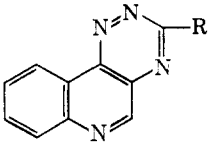
**Table II.** Triazinoquinolines. Antifungal Activity



| No. | R | MIC[a] in Sabouraud's dextrose broth: *T. glabrata* (VM-22)[b] | *C. tropicalis* (VM-25)[b] | *C. krusei* (VM-29B)[b] | *C. guilliermondi* (VM-42)[b] | *C. albicans* (VM-71)[b] | *C. albicans* (VM-81)[b] | *C. albicans* (M-3)[b] |
|---|---|---|---|---|---|---|---|---|
| **1** | Cl | ≦10 | ≦10 | ≦10 | ≦10 | ≦10 | ≦10 | ≦10 |
| **2a** | $NH_2$ | 30 | >50 | 10 | >50 | 30 | 40 | 30 |
| **2b** | $NHCH_2CH_2OH$ | 30 | >50 | 20 | >50 | 30 | 50 | 40 |
| **2c** | $N(CH_2CH{=}CH_2)_2$ | 40 | >50 | 20 | 30 | 40 | 50 | 50 |
| **3** | $OCH_3$ | 40 | 40 | 20 | 30 | 40 | 40 | 20 |
| Nystatin[c] | | ≦10 | ≦10 | ≦10 | ≦10 | ≦10 | ≦10 | ≦10 |

[a]Minimal inhibitory concentration, μg/ml, dissolved in *N,N*-dimethylacetamide (ref 4). [b]Norwich Pharmacal culture number. [c]Potency = 4162 units/mg.

hr. The resultant bright yellow solid was collected by filtration and washed with 95% EtOH (MeOH) (3 × 50 ml), *i*-PrOH (3 × 50 ml), and $Et_2O$ (3 × 250 ml); yield 190 g (theory, 141 g of **7** plus 37 g of NaCl).

***as*-Triazino[5,6-*c*]quinolin-3(4*H*)-one (9). Method A.** To AcOH (760 ml) at 15–20° was added **7** (with residual NaCl) (190 g) with mechanical stirring. The reaction mixture was allowed to warm to room temperature in 2 hr and then cooled to 17° for 2 hr. A yellow, crystalline solid was collected by filtration and washed with *i*-PrOH (5 × 100 ml) and $Et_2O$ (5 × 200 ml): mp 305–313° dec; yield 158 g (theory, 129 g plus 37 g of NaCl). Recrystallization three times from AcOH gave **9**: mp 284–294° dec; ir (Nujol) 5.93 μ (C=O). *Anal.* ($C_{10}H_6N_4O$) C, H, N.

**Method B.** A mixture of **8** (0.6 g) and AcOH (20 ml) was treated with $Pb(OAc)_4$ (4.0 g), and the reaction mixture was stirred at room temperature for 1 hr. The resultant solid was collected by filtration and washed with AcOH, *i*-PrOH, and $Et_2O$. Recrystallization from AcOH gave **9**; the infrared absorption was identical with **9** prepared by method A.

**3-Chloro-*as*-triazino[5,6-*c*]quinoline (1).** Compound **9** (35 g, 0.16 mol) was added to $POCl_3$ (150 ml) at 20–25° with rapid stirring. The mixture was heated to 100° in 10 min, maintained at 102–105° for 7 min, and then cooled rapidly to 2–5°. The cooled reaction mixture was poured into ice (1300 g) with rapid stirring, allowed to warm to 15° over 1 hr, and then cooled an additional 1 hr in an ice bath. Solid product was collected by filtration and washed with $H_2O$ (5 × 80 ml). The air-dried product was further dried in a vacuum desiccator over Drierite, mp 171–181° dec; recrystallization from EtOAc gave **1**.

**3-Amino-*as*-triazino[5,6-*c*]quinoline (2a).** A mixture of **1** (10 g, 0.05 mol) and 95% EtOH (MeOH) (190 ml) was treated with a stream of dry $NH_3$ for 45 min at 35–45°, with rapid stirring. The reaction mixture was cooled in an ice bath for 1 hr, and the resultant bright yellow, crystalline solid was collected by filtration and washed with 95% EtOH ($Et_2O$) (6 × 10 ml) and $Et_2O$. An analytical sample was prepared by recrystallization from 90% DMF (MeOH).

Compounds **2b** and **2c** were prepared by the same procedure as described for that of **2a**.

**3-Methoxy-*as*-triazino[5,6-*c*]quinoline (3).** A mixture of **1** (44 g, 0.20 mol) and a solution of NaOMe (11 g, 0.20 mol) in MeOH (2000 ml) was refluxed for 1 hr and then filtered hot. The cooled filtrate was filtered to give **3**, mp 163–167°. An analytical sample was prepared by recrystallization from MeOH.

**Acknowledgments.** The authors are grateful to Mr. Nelson Miles for assistance in the preparation of compounds, to Mr. Grant Gustin and Mr. Marvin Tefft for the elemental analyses, and to Mr. Ralph Bush and Mr. Larry Wood for their assistance in the antifungal studies.

## References

(1) G. C. Wright and C. N. Yu, U. S. Patent 3,721,670 (1973).
(2) J. B. Polya and G. F. Shanles, *J. Chem. Soc.*, 4986 (1964).
(3) G. B. Bachman, D. E. Welton, G. L. Jenkins, and J. E. Christian, *J. Amer. Chem. Soc.*, **69**, 368 (1947).
(4) D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics: A Laboratory Manual," Medical Encyclopedia, New York, N. Y., 1955.
(5) G. F. Reddish, "Antiseptics, Fungicides and Chemical and Physical Sterilization," 1st ed, Lea and Febiger, New York, N. Y., 1954, pp 135–136.

## Radiopharmaceuticals. 12. A New Rapid Synthesis of Carbon-11 Labeled Norepinephrine Hydrochloride†

Joanna S. Fowler,* Robert R. MacGregor, Azizullah N. Ansari, Harold L. Atkins, and Alfred P. Wolf

*Brookhaven National Laboratory, Upton, New York 11973.*
*Received July 30, 1973*

The rapid growth in the use of radiopharmaceuticals for external radioscanning has resulted in an increasing need for new safer radiopharmaceuticals labeled with elements emitting radiation which can be detected outside the body barrier. The potential impact of organ-specific radiopharmaceuticals labeled with short-lived nuclides on the safety of diagnostic procedures has justified expending considerable effort in the development of rapid organic synthetic procedures in order to accomplish the incorporation of simple labeled precursors into relatively complex molecules of pharmacological interest.[1,2]

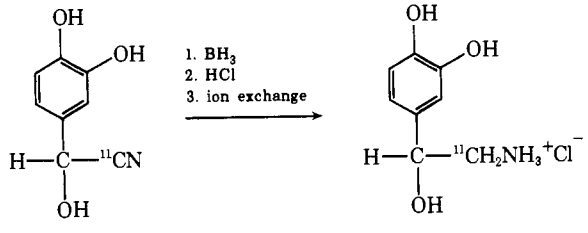
One of the more potentially useful radionuclides for labeling is $^{11}C$ which decays by positron emission and has a half-life of 20.4 min. The advantage of $^{11}C$ for diagnostic procedures lies in its short half-life (which lowers the radiation dose to the patient) and positron emission (which offers improved resolution on scanning by detection of the 511-keV annihilation radiation). In addition, since carbon is naturally occurring the properties of the normal bioactive molecules are not significantly altered by labeling. However, the reduced time scale in which one must work, as well as the limited number of readily available $^{11}C$-labeled precursor molecules[1] (for example $^{11}CO$, $^{11}CO_2$, $^{11}CH_2O$, $H^{11}CN$, $H^{11}C \equiv {}^{11}CH$, etc.), presents certain restrictions on the nature of the $^{11}C$-labeled radiopharmaceuticals which one can prepare employing syntheses described in the chemical literature.

Presently there is considerable interest in developing an agent which would localize in the myocardium and thus aid in the diagnosis of myocardial infarction. Tracer studies with both $^{14}C$ and $^{3}H$ have shown that norepinephrine rapidly localizes in heart tissue.[3,4] This suggested that

†Research performed under the auspices of the U. S. Atomic Energy Commission.

norepinephrine labeled with $^{11}C$ would be potentially useful as a myocardial scanning agent.

The known literature methods for synthesizing norepinephrine were not applicable to labeling this molecule with $^{11}C$. The availability of carrier-free $H^{11}CN$ in our laboratory[5] as well as the fact that we could use this material to prepare cyanohydrin-$^{11}C$ (I), an intermediate in the dopamine-$^{11}C$ synthesis, made a synthetic route using I particularly attractive.[6] A search of the literature revealed that the reduction of this cyanohydrin to norepinephrine was previously reported using sodium amalgam, a reaction which failed in our hands.[7] The common methods of reducing cyanohydrins to β-amino alcohols are catalytic hydrogenation or chemical reduction with lithium aluminum hydride. Catalytic hydrogenation could not be used in this case because of the known hydrogenolysis of benzylic alcohols.[8] Chemical reduction with lithium aluminum hydride was unsuccessful, probably due to the presence of the catechol group, which formed highly insoluble complexes with the reducing agent, and to the instability of the substrate to oxidation in a basic medium. Because of the problems of decomposition in basic media the use of borane ($BH_3$), a Lewis acid reducing agent,[9] was investigated. It was found that the reduction of the cyanohydrin (I) proceeds rapidly (5 min) at room temperature to give norepinephrine hydrochloride (II). The entire procedure requires only 40 min and an overall chemical yield of 10–15% (based on 3,4-dihydroxybenzaldehyde) is routinely obtained. To our knowledge, borane has not been used previously to carry out the reduction of a cyanohydrin to a β-amino alcohol. This is a very special case of the use of $BH_3$ to reduce a cyanohydrin which cannot be reduced to a β-amino alcohol by the known literature methods. However, since solutions of borane are commercially available and since the reaction proceeds very rapidly, its use could probably be extended to other systems. This procedure is readily adaptable to producing *carrier-free* norepinephrine-*l*-$^{11}C$ hydrochloride.




I II

The development of routes to carrier-free $^{11}C$-labeled radiopharmaceuticals is important for two reasons. Firstly, striking loading dose effects in organ uptake with both 6-fluorotryptophan-$^{18}F$[10] and dopamine-$^{11}C$[11] have been observed, thus emphasizing the importance of developing methods of producing carrier-free radiopharmaceuticals so that organ localization as a function of loading dose can be studied. Secondly, with carrier-free $^{11}C$ radiopharmaceuticals an amount of radioactivity sufficient for a scan represents a physiologically insignificant quantity of pharmaceutical (20 mCi of carrier-free noradrenaline-*l*-$^{11}C$ hydrochloride = 0.43 ng). Therefore, dynamic studies of the metabolism of biologically active molecules can be made at the true tracer level without a drug effect from the administered tracer. This is not usually the case with $^{14}C$-labeled molecules since a much greater dose must be administered in order to obtain significant counting statistics.

The usefulness of carrier-free $^{11}C$-labeled norepinephrine hydrochloride is presently being evaluated in animals. In a preliminary experiment, four dogs were given norepinephrine-$^{11}C$ hydrochloride intravenously and the uptake by different tissues at different times was studied. The dogs were sacrificed at 15, 30, 60, and 90 min postinjection. The per cent uptake per gram of the administered dose in the heart at 15, 30, 60, and 90 min was 0.017, 0.023, 0.025, and 0.023, respectively. The ratio of heart to blood, lung, liver, kidney, and adrenal medulla at 1 hr was 11.04, 4.89, 2.28, 0.98, and 0.28, respectively. The biological half-life of norepinephrine-$^{11}C$ hydrochloride in the blood is less than 2 min. Imaging experiments as well as more detailed tissue distribution experiments are in progress and will be reported later.

## Experimental Section

**Norepinephrine Hydrochloride.** To 4 mg (0.0816 mmol) of NaCN in 0.05 ml of water was added 10 mg (0.0725 mmol) of 3,4-dihydroxybenzaldehyde and 9 mg (0.0865 mmol) of sodium bisulfite which had been previously dissolved in 0.1 ml of water. This solution was heated at 70° for 3 min and extracted with ether, and the ether solution was filtered through $CaCl_2$. The ether solution was blown to dryness using a stream of nitrogen. To the residue was added 0.1 ml of dry ether and 0.2 ml of 1 *M* borane solution (Alfa Inorganics). This solution was allowed to stand 5 min at room temperature, the ether was removed using a stream of nitrogen, and 0.2 ml of 1 *M* HCl and 1 ml of water were added. This was rinsed onto a column (0.5 × 2.0 cm) of Dowex 50 W-X8 (prewashed according to the procedure described in ref 12) using ~4 ml of water and the column was rapidly washed with 20 ml of 0.001 *M* HCl which was discarded. The norepinephrine hydrochloride was washed from the column with 15 ml of 3 *M* HCl. The solvent was removed *in vacuo*. Tlc (silica gel) with 1-butanol, acetic acid, and water (15:3:5) showed only norepinephrine hydrochloride ($R_f$ 0.5) which could be detected by uv, potassium ferricyanide solution, or ninhydrin. An ir (KBr) of the residue was identical with that of authentic norepinephrine hydrochloride. The yield was 14.2% as determined by uv [$\lambda_{max}$ (0.1 *M* HCl) 278 nm (ε 2990)].

**Norepinephrine-*l*-$^{11}C$ Hydrochloride (Carrier-Free).** Carrier-free $^{11}C$-labeled HCN was transferred on a vacuum line into 0.05 ml of 0.05 *M* NaOH; the solution was stirred at room temperature for 1 min and evaporated for dryness. To the residue of carrier-free $Na^{11}CN$ was added 5 mg (0.0362 mmol) of 3,4-dihydroxybenzaldehyde and 4.5 mg (0.0432 mmol) of sodium bisulfite which had previously been dissolved in 0.05 ml of water. The procedure as described for the unlabeled product was then followed to yield $^{11}C$-labeled norepinephrine hydrochloride. The chemical integrity of the labeled material was confirmed by tlc in three different solvent systems [1-butanol–acetic acid–water (15:3:5); 1-butanol–pyridine–acetic acid–water (15:2:3:5); ethyl acetate–acetic acid–water (15:15:10)] with carrier norepinephrine hydrochloride added. All of the radioactivity was congruent with the norepinephrine hydrochloride.

Radiochemical yields are approximately 10% based on the initial activity of $H^{11}CN$ delivered. Thus, with 1.5 Ci of $H^{11}CN$ at the end of bombardment 37.5 mCi of $^{11}C$-labeled norepinephrine hydrochloride is delivered for animal experiments.

## References

(1) A. P. Wolf, D. R. Christman, J. S. Fowler, and R. M. Lambrecht, New Developments in Radiopharmaceuticals and Labeled Compounds, Copenhagen, 1973 (IAEA/SM-171).
(2) H. I. Glass, Symposium on Medical Radioisotope Scintigraphy, Monaco, 1972 (IAEA/SM-164/305).
(3) O. Leder and A. Fleckenstein, *Nucl. Med., Suppl.*, **2**, 423 (1963).
(4) C. Su, J. A. Bevan, and J. V. Osher, *Experientia*, **28**, 991 (1972).
(5) D. R. Christman, R. D. Finn, K. I. Karlstrom, and A. P. Wolf, *J. Nucl. Med.*, **14**, 864 (1973).
(6) D. R. Christman, R. M. Hoyte, and A. P. Wolf, *J. Nucl. Med.*, 11, 474 (1970).
(7) Meister Lucius & Brüning, Hochst A. M., German Patent Nr. 193,634; *Chem. Zentralbl.*, Pt. 1, 430 (1908).
(8) W. H. Hartung, *Org. React.*, **7**, 263 (1953).
(9) H. C. Brown, "Hydroboration," W. A. Benjamin, New York, N. Y., 1962, Chapter 17.
(10) H. L. Atkins, D. R. Christman, J. S. Fowler, W. Hauser, R.

M. Hoyte, J. F. Klopper, S. S. Lin, and A. P. Wolf, *J. Nucl. Med.*, **13**, 713 (1972).
(11) J. S. Fowler, A. N. Ansari, H. L. Atkins, P. R. Bradley-Moore, R. R. MacGregor, and A. P. Wolf, *J. Nucl. Med.*, **14**, 867 (1973).
(12) J. Häggendahl, *Scand. J. Clin. Lab. Invest.*, **14**, 537 (1962).

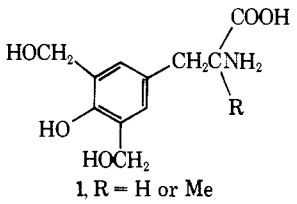
## Saligenin Analogs of *l*-Dopa and *dl*-α-Methyl-Dopa

Michael Atkinson, David Hartley,* Lawrence H. C. Lunts, and Alexander C. Ritchie

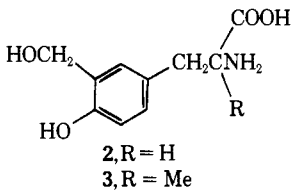
*Chemistry Department, Allen and Hanburys Research Ltd., Ware, Hertfordshire, England.* *Received July 12, 1973*

We have described[1] some saligenin derivatives that retain the adrenergic activity of the corresponding catechols and the discovery of a long-acting and highly selective bronchodilator [salbutamol (Ventolin)]. It seemed worthwhile to modify other biologically active catechols in a similar fashion and to study their properties. We now wish to report the preparation of saligenin analogs of Dopa and α-methyl-Dopa as potential drugs for the treatment of Parkinson's disease and hypertension, respectively.

These syntheses were achieved by hydroxymethylation of the *N*-benzyloxycarbonyl derivatives of the corresponding tyrosines, using HCHO and NaOH at room temperature. The protecting group was removed by catalytic hydrogenolysis, using Pd black in the presence of $NH_4OH$ to conserve the saligenin function. The products from reactions with 1 molar equiv of HCHO were shown by thin-layer and paper chromatography to be mixtures containing unchanged starting materials and bishydroxymethylated compounds **1** in addition to the saligenins **2** or **3**. The bishydroxymethylated compounds were identified by singlet peaks in their nmr spectra ($D_2O$–NaOH) at δ 4.62 (4 H, $CH_2OH$) and 6.97 (2 aromatic protons). Even when the amount of HCHO was reduced to between 0.8 and 0.2 molar equiv there was always a preponderance of the bishydroxymethylated product **1** over the desired mono derivative **2** or **3**. This problem was overcome by adding $Na_2B_4O_7$ to chelate with the saligenin moiety of the initial product. Thus, by binding the phenolic group, further nuclear substitution is prevented and the required amino acids **2** and **3** were obtained in overall yields of greater than 25%.



**1**, R = H or Me



**2**, R = H
**3**, R = Me

The hydroxymethylation step required 14–20 days at room temperature and was difficult to force to completion. Hydrogenolysis of the product from reactions using shorter times always gave some unchanged *l*-tyrosine or *dl*-α-methyltyrosine, although these materials could be removed by preferential crystallization from $H_2O$.

Neither compound *l*-**2** nor *dl*-**3** lowered the systolic blood pressure of the DOCA hypertensive rat[2] in doses of 50–200 mg/kg given subcutaneously each day for 3 days, whereas *l*-α-methyl-Dopa produced a fall of 35 mm after a single dose of 20 mg/kg and of 60 mm after three doses of 20 mg/kg given over 2 days. Using the procedure described by Maj, *et al.*,[3] both *l*-Dopa and the analog **2** at doses of 250 mg/kg, ip, reversed the catalepsy induced in rats by ip administration of 0.5 mg/kg of haloperidol, a blocker of dopaminergic receptors. However, this was the only test that suggested biological similarity. In other tests in which *l*-Dopa is effective, such as the enhancement of circulatory behavior of rats with a unilateral lesion in the nigrostriatal system,[4] or the antagonism of tremor induced in rats by harmine,[5] the saligenin **2** was inactive in doses of 250 mg/kg, ip.

From these results it seems unlikely that the compounds would be of value in the treatment of hypertension or Parkinson's disease.

The absence of the desired activities in **2** and **3** may be related to their lack of significant interaction with Dopa decarboxylase. Thus, they did not inhibit kidney Dopa decarboxylase from the guinea pig or rat at a concentration of $1.54 \times 10^{-3}$ *M* ($ID_{50}$ of *dl*-α-methyl-Dopa is $7 \times 10^{-6}$ *M*).[6] Neither were the compounds substrates (for the experimental procedure see ref 7) at the same concentration ($K_m$ of Dopa is $1.7 \times 10^{-3}$). These *in vitro* results might also explain why no evidence could be found for depletion of levels of norepinephrine in mouse hearts,[8] as little of the corresponding dopamines would be formed to displace norepinephrine from its stores.

### Experimental Section

Melting points were determined on a Townson and Mercer melting point apparatus and the microanalyses on an F & M 185 C, H, and N analyzer. Where analyses are indicated by the symbols of the elements, analytical values were within ±0.4% of the calculated values. Compounds gave satisfactory uv, ir, and nmr spectral data obtained respectively on Perkin-Elmer Model 137, Unicam SP 100, and Varian Associates A-60A spectrometers. Paper and tlc chromatograms were run on Whatman No. 1 paper (BuOH–AcOH–$H_2O$, 60:15:25) and $SiO_2$ (EtOAc–MeOH, 1:1), respectively, and the products identified by staining blue with 1% $FeCl_3$ solution.

*l*-*N*-**Benzyloxycarbonyl-3-(hydroxymethyl)tyrosine.** A solution of $Na_2B_4O_7$ (14 g) in $H_2O$ (200 ml) and 36% HCHO (10 ml, 0.1 mol) was added to *l*-*N*-benzyloxycarbonyltyrosine (10 g, 0.03 mol) in 1 *N* NaOH (60 ml). After 14 days at room temperature no starting material could be detected by tlc. The pH was adjusted to between 1 and 2 with 1 *N* HCl to deposit a white oil which was extracted into EtOAc. The extracts were washed with brine, dried ($MgSO_4$), and evaporated to give a clear oil which solidified on standing: 9 g; mp 125–129°. Two recrystallizations from EtOAc gave *l*-*N*-benzyloxycarbonyl-3-(hydroxymethyl)tyrosine as a white powder: 6.9 g (63%); mp 131.5–133°; $[\alpha]^{24.5}D$ +10.4° (*c* 0.75, AcOH). *Anal.* ($C_{18}H_{19}NO_6$) C, H, N.

*l*-**3-(Hydroxymethyl)tyrosine (2).** A solution of *l*-*N*-benzyloxycarbonyl-3-(hydroxymethyl)tyrosine (2.2 g, 0.0064 mol) in EtOH (30 ml) and 1 *N* $NH_4OH$ (7 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of Pd black (0.5 g). Uptake of $H_2$ (0.0056 mol) ceased after 1 hr. The catalyst and solvent were removed to leave the amino acid as a white powder: 1.2 g (88%); mp >300°; $[\alpha]^{24}D$ −9.2° (*c* 0.38, 0.1 *N* HCl) *Anal.* ($C_{10}H_{13}NO_4 \cdot 0.25H_2O$) C, H, N.

*dl*-**3-(Hydroxymethyl)-α-methyltyrosine (3).** *dl*-α-Methyltyrosine[9] reacted with benzyl chloroformate and NaOH by a standard procedure[10] to give *dl*-*N*-benzyloxycarbonyl-α-methyltyrosine in 65% yield: mp 183–184°. *Anal.* ($C_{18}H_{19}NO_5$) C, H, N. The latter (6 g, 0.018 mol) was hydroxymethylated in a manner similar to that described for the corresponding tyrosine derivative and the crude product hydrogenated to give *dl*-3-(hydroxymethyl)-α-methyltyrosine (**3**): 1.8 g (41%); mp >300°. *Anal.* ($C_{11}H_{15}NO_4$) C, H, N.

**Acknowledgment.** We thank our colleagues Dr. R. T. Brittain, Mr. L. E. Martin, and Dr. J. Hunt and their staff for pharmacological, biochemical, and analytical data, respectively, and Mr. M. T. Davies of BDH (Research), Ltd., London, for the optical rotations. We are especially grateful to Dr. R. J. Naylor of the University of Bradford for carrying out the pharmacological tests to assess the potential of some of the compounds in Parkinson's disease.