Synthesis of ^{14}C-Labeled Bunolol

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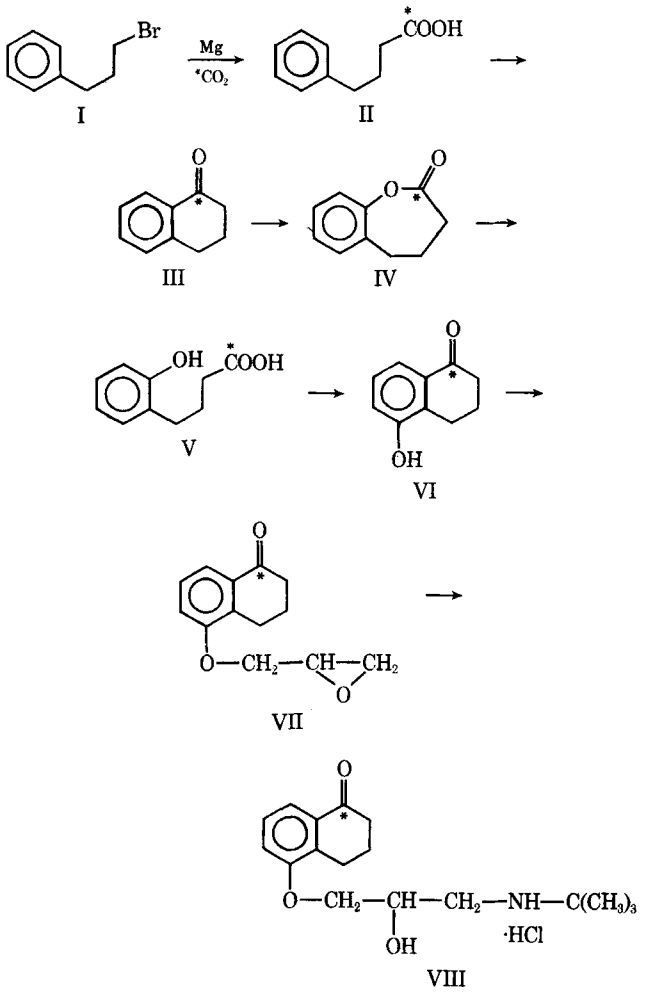
Abstract □ Ring-labeled bunolol, *dl*-5-[3-(*tert*-butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2*H*)-naphthalenone-1-^{14}C, was synthesized in several steps by initially allowing 3-phenylpropylmagnesium bromide to react with $^{14}CO_2$. The resulting 4-phenylbutyric-1-^{14}C acid was cyclized to α-tetralone-1-^{14}C. The ring was then oxidized and opened to 4-(2-hydroxyphenyl)butyric-1-^{14}C acid, which, when recyclized, gave 5-hydroxytetralone-1-^{14}C The 5-hydroxytetralone-1-^{14}C was allowed to react, sequentially, with epichlorohydrin, *tert*-butylamine, and gaseous hydrogen chloride to give bunolol hydrochloride. After purification, a number of physical tests established the chemical and radiochemical purity of this material.

Keyphrases □ Bunolol, ^{14}C-labeled—synthesis □ *dl*-5-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2*H*)-naphthalenone-1-^{14}C (bunolol)—synthesis

Bunolol hydrochloride, *dl*-5-[3-(*tert*-butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2*H*)-naphthalenone hydrochloride (VIII), is a new β-adrenergic blocking agent whose cardiovascular pharmacology was reported by Robson *et al.* (1). The drug was labeled with ^{14}C to facilitate determining its absorption and excretion rates, blood levels, tissue distribution, and biotransformation (2). The synthesis, shown in Scheme I, was designed to incorporate a ^{14}C atom into position 1 of the naphthalenone ring to assure retention of the label during metabolism.

The preparation of 4-phenylbutyric-1-^{14}C acid (II) was modified from Rupe and Proske's (3) procedure to allow for the handling of radioactive materials in conventional apparatus as described by Collins (4). The cyclization to α-tetralone-1-^{14}C (III) and its oxidation, ring opening, and recyclization to 5-hydroxy-α-tetralone-1-^{14}C (VI) were adapted to allow for the handling of small quantities from the procedures of Pattison and McMillan (5), which were based on the work of Schroeter (6). The subsequent conversions were modified from those of Schwender *et al.* (7).

The overall chemical yield was 7.3%, while the radiochemical conversion was 6.4%. The physical constants, which established that VIII was 99% chemically and radiochemically pure, are included in the *Experimental* section.



Scheme I

EXPERIMENTAL

4-Phenylbutyric-1-^{14}C Acid (II)—Three flame-dried, three-necked, round-bottom flasks were connected in series, with equilibrated addition funnels in the first two flasks, a $CaSO_4$ drying tube between the second and third flasks, and two reflux condensers and a rubber septum fitted to the third flask.

Into the first flask, 0.7 g. Ba-$^{14}CO_3$ (100.8 mc., specific activity 145 mc./g.)[1] was added, and 30 ml. of 5 *N* perchloric acid (previously degassed) was added to the addition funnel. Into the second flask, 4.24 g. (25 mmoles total) of $BaCO_3$ was added, and 220 ml. of 5 *N* perchloric acid (previously degassed) was added to the addition funnel. To the third flask, 4.98 g. (25 mmoles) of 3-phenylpropyl bromide and 0.7 g. (28.8 mmoles) of ether-washed magnesium turnings were added. The entire system was flushed with nitrogen gas and then kept at atmospheric pressure. To the third flask, 250 ml. of anhydrous ether, dried over Safe-Na[2], was injected through the septum using a hypodermic syringe. The reaction was magnetically stirred and allowed to reflux gently for 2 hr. During this time, the magnesium turnings reacted completely. This flask was cooled to −45° using a mixture of 150 parts carbon tetrachloride and 20 parts chloroform over dry ice (4). The pressure was reduced with an oil pump to about 12 mm., as measured with a mercury-filled manometer, and the system was closed.

The perchloric acid was allowed to drip slowly onto the magnetically stirred Ba-$^{14}CO_3$. When all the $^{14}CO_2$ was liberated, the unlabeled $BaCO_3$ was decomposed. The liberation of CO_2 under these conditions was quantitative, and the uptake was rapid. The progress of the reaction was readily followed by the initial rise and then drop in pressure observed after each addition. After all of the CO_2 was liberated, the reaction was allowed to stir at −45° for an additional 30 min., and the cooling bath was removed. When room temperature was attained, a pump[3] was used to transfer the un-

[1] Mallinckrodt/Nuclear, St. Louis, Mo.

[2] J. T. Baker Chemical Co., Phillipsburg, N. J.

[3] Toepler pump, Roder Instrument Co., Los Altos, CA 94022

reacted $^{14}CO_2$ into a flask containing a magnetically stirred, filtered solution of 16.8 g. of $Ba(OH)_2 \cdot 8H_2O$ in 300 ml. of distilled water.

The third flask, containing the Grignard complex, was cooled to −20°; 50 ml. of 6 *N* aqueous hydrochloric acid was added slowly. The phases were separated, and the aqueous phase was extracted with one 50-ml. portion of ether. The ether phases were combined and extracted with six 50-ml. portions of 1 *N* aqueous sodium carbonate. The combined aqueous phases were washed with 50 ml. of ether and then acidified by adding 75 ml. of 6 *N* aqueous hydrochloric acid. This was extracted with four 75-ml. portions of ether, which were combined, dried ($MgSO_4$), and filtered; the solvent was removed to give 1.50 g of 4-phenylbutyric-1-^{14}C acid.

The flask containing the trapped Ba-$^{14}CO_3$ was refluxed for 5–10 min. to coagulate the precipitate; it was then cooled and filtered. After drying, the recovered Ba-$^{14}CO_3$ weighed 2.5 g. (58.4% recovery), which was recycled in the above reaction, making the necessary molar adjustments. This procedure gave an additional 1.21 g. of 4-phenylbutyric-1-^{14}C acid, or a total of 2.71 g. (66%) plus the recovery of 0.3 g. of Ba-$^{14}CO_3$.

The 4-phenylbutyric-1-^{14}C acid had a specific activity[4] of 4.51 mc./mmole (theory = 4.31 mc./mmole) and was sufficiently pure to prepare III.

α-Tetralone-1-^{14}C (III)—A mixture of 2.91 g. (49.7 mmoles) of sodium chloride and 14.65 g. (109.7 mmoles) of aluminum trichloride, after flushing with nitrogen, was mechanically stirred, and the flask was introduced into a preheated oil bath at 100°. The oil bath temperature was raised to about 170° over 30 min. During this time, a dark-amber melt resulted. The oil bath was removed, but the reaction was allowed to stir until the internal temperature dropped to 110°. The 2.71 g. (16.5 mmoles) of 4-phenylbutyric-1-^{14}C acid was added in one portion, the stirring was continued, and the flask was reimmersed into the oil bath at about 145°. The temperature of the oil bath was raised to 200° over 30 min. and kept at this temperature for an additional 15 min. The black mass was cooled in an ice bath, and 33 ml. of benzene was added. The complex was decomposed by the portionwise addition of 73.9 g. of ice and 7.39 ml. of concentrated hydrochloric acid. After about 45 min. of stirring, no solids remained.

The phases were separated, and the aqueous phase was extracted with six 15-ml. portions of benzene. The benzene extracts were combined, protected from light, and dried overnight with magnesium sulfate and about 1 g. of powdered sodium carbonate. The solids were filtered, and the solvent was removed. The residual brown oil was distilled; the fraction boiling at 133°/15 mm. was collected. The α-tetralone-1-^{14}C, a pale-yellow oil, weighed 1.46 g. (61%) and was used to prepare V.

4-(2-Hydroxyphenyl)butyric-1-^{14}C Acid (V) (8)—To a magnetically stirred solution of 1.46 g. (10 mmoles) of α-tetralone-1-^{14}C in 7 ml. methanol, containing 0.54 ml (30 mmoles) of water cooled to −12° in a bath of *tert*-amyl alcohol and dry ice, was added a slurry of 4.50 g. (15 mmoles) of finely ground potassium persulfate in 3.33 ml. (60 mmoles) of concentrated sulfuric acid. The persulfate–sulfuric acid slurry was magnetically stirred and added portionwise over about 1 hr., keeping the internal temperature of the reaction between 0 and 5°. After the addition was complete, the bath was replaced with an ice bath, and the reaction was allowed to stir an additional 2 hr. at 0–5°.

The dark mass was poured over 10 g. of ice and extracted with three 25-ml. portions of chloroform. The organic phases were combined and extracted with three 10-ml. portions of 1 *N* aqueous sodium hydroxide. The combined basic extracts were allowed to reflux for 15 min., cooled, and acidified with 10 ml. of 6 *N* hydrochloric acid. This mixture was extracted with three 25-ml. portions of chloroform after the addition of 10 g. of sodium chloride. The organic phases were combined, dried ($MgSO_4$), and filtered; then the solvent was removed. The residual oil solidified when scratched to give 1.2 g. (67%) of 4-(2-hydroxyphenyl)butyric-1-^{14}C acid, which was used to prepare VI.

5-Hydroxy-α-tetralone-1-^{14}C (VI)—The cyclization of 1.2 g. (6.7 mmoles) of 4-(2-hydroxyphenyl)butyric-1-^{14}C acid was accomplished in the same manner as described for the cyclization of II to yield III. A mixture of 1.17 g. (20 mmoles) of sodium chloride and 5.93 g. (44.5 mmoles) of aluminum trichloride was used.

The reaction was allowed to decompose by slowly adding 45 g. of ice and 4.5 ml. of concentrated hydrochloric acid to the dark mass. It was necessary to break up some of the solids mechanically. After stirring for 45 min., the insolubles were filtered. The cake was triturated with 10 ml. of water, refiltered, and washed with two 5-ml. portions of water. The solid weighed 1.09 g. (100%) after drying in a pistol for 4 hr. at 61° at a pressure of 0.1 mm. The crude 5-hydroxy-α-tetralone-1-^{14}C was used to prepare VII.

5-(2,3-Epoxypropoxy)-3,4-dihydro-1(2*H*)-naphthalenone-1-^{14}C (VII)—To a solution of 1.09 g. (6.7 mmoles) of 5-hydroxy-α-tetralone-1-^{14}C in 10.9 ml. of absolute ethanol and 1.35 ml. of 6 *N* sodium hydroxide was added 9.29 g. (100 mmoles) of epichlorohydrin in one portion. This mixture was vigorously stirred at room temperature for 24 hr. The reaction was filtered, the insolubles were washed with three 2-ml. portions of ethanol, and the solvents were removed from the combined filtrates.

The brown residue was partitioned between 16.5 ml. of water and 16.5 ml. of chloroform. The aqueous phase was extracted with an additional three 16.5-ml. portions of chloroform. The combined organic phases were washed with two 7-ml. portions of water, dried ($MgSO_4$), and filtered; then the solvent was removed. The brown oil was further extracted with ten 10-ml. portions of anhydrous ether. The solvent was removed from the combined ether phases, and the residue was distilled. That fraction boiling at 132–134°/0.1 mm. weighed 0.74 g. (51%); it was a pale-yellow oil. This 5-(2,3-epoxypropoxy)-3,4-dihydro-1(2*H*)-naphthalenone-1-^{14}C was used to prepare VIII.

***dl*-5-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-3,4-dihydro-1(2*H*)-naphthalenone-1-^{14}C Hydrochloride (VIII)**—To a solution of 0.74 g. (3.4 mmoles) of VII in 7.4 ml. of ethanol was added 1.24 g. (17 mmoles) of *tert*-butylamine, and the solution was allowed to reflux for 45 min. The solvent was removed, and the residue solidified when scratched. The solid was triturated with 8 ml. of cold cyclohexane. The off-white solid, when filtered, gave 0.7 g. (71%) of the free base of VIII.

This was allowed to dissolve in 3.5 ml. of chloroform and was converted into the hydrochloride salt by allowing gaseous hydrogen chloride to bubble through the magnetically stirred solution for about 15 min. The addition of 3.5 ml. of anhydrous ether precipitated the salt, which, after filtering, was triturated with 6 ml. of cold cyclohexane. The solid was recrystallized by dissolving in 1.4 ml. boiling absolute ethanol, which was filtered; two portions of 0.35 ml. of anhydrous ether were added to the filtrate. The precipitate, when filtered, gave 0.60 g. (53.8%) of VIII. The specific activity[4] was determined to be 4.43 mc./mmole (13.5 mc./g.). This product was diluted by allowing a mixture of 0.6 g. of synthesized VIII and 1.0 g. of authentic VIII to recrystallize from 16 ml. of boiling absolute ethanol, to which was added two 4-ml. portions of anhydrous ether. The precipitate, when filtered, gave 1.3 g. of VIII which melted at 225–227°.

A UV spectrum was determined in ethanol and exhibited maxima at 221 and 254 nm., identical to an authentic sample. The absorptivity at these maxima was 72.8 and 25.9, respectively, which was 99.6% of the reference standard. An IR spectrum was determined as a mull and exhibited maxima only at the same frequencies as an authentic sample. TLC on a glass plate coated with 250 μ of silica gel GF, spotted with 200 mcg. of material and irrigated with a mixture of 9 parts chloroform (saturated with ammonium hydroxide) and 1 part methanol[6], showed the main UV-absorbing spot at an R_f of about 0.55 (identical with an authentic sample) and traces at about 0.83 (about 0.5%) and at the origin (about 0.25%). When scanned for radioactivity[6], the main spot contained 99.0% of the radioactivity on the plate, while the faster moving spot contained 0.75% and the spot on the origin contained 0.25%. This faster moving contaminant was identified as either *dl*-5,5′-[3,3′-(*tert*-butylamino)bis(2-hydroxypropoxy)]-bis[3,4-dihydro-1(2*H*)-naphthalenone]hydrochloride or the analogous *meso*-compound[7].

A gas chromatogram[5], using a 1.82-m. (6-ft.) glass column packed with 4% OV-17 on 80/100 mesh Gas Chrom Q (operated at 188°

[4] A model 3310 Packard Tri-Carb liquid scintillation spectrometer, equipped with automatic external standardization, was used. The cocktail was composed of 7.0 g. 2,5-diphenyloxazole, 0.3 g. 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene, and 100 g. naphthalene dissolved in 1 l. 1,4-dioxane.

[5] This system was developed by A. D. Lewis and M. Goodenough of these laboratories.

[6] A model 7200 Packard radiochromatogram scanner was used.

[7] This compound was synthesized by R. Novack of these laboratories.

with a helium gas flow rate of 55 ml./min.) and equipped with a flame detector, gave a single peak with an R_t of 34 min.

The specific activity was determined[4] to be 1.61 mc./mmole (4.90 mc./g.).

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Effect of Formulation of Anagestone Acetate on Progestational Proliferation of Rabbit Uterus after Oral Administration

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Abstract □ A method is presented for the determination of *in vitro* dissolution rates for anagestone acetate, which yields results representative of *in vivo* bioavailability in animal studies. This procedure appeared to be satisfactory in preliminary screening of tablet formulations of anagestone acetate.

Keyphrases □ Anagestone acetate—formulation effects of progestational proliferation, rabbit uterus, oral administration □ Dissolution rates, *in vitro*, anagestone acetate—correlated to *in vivo* bioavailability, rabbits □ Formulation, effect on progestational proliferation, rabbit uterus—preliminary tablet screening of anagestone acetate

Even though tablet disintegration time is widely used, it has inherent defects which limit its usefulness in screening tablet formulations. Various reviews (1, 2) emphasized that the length of time required for a tablet to disintegrate *in vitro* cannot be taken as a direct indication of the time required for *in vivo* availability. Much of the literature suggests that better correlations are obtained using *in vitro* dissolution-rate studies; however, no single dissolution test can be applied to all drugs. The possibility that a single dissolution test can be applied to drugs having similar physicochemical properties remains to be established.

In an effort to develop tablet formulations containing anagestone acetate[1] that would allow ease of formulation and yet ensure maximum bioavailability, a correlation has been utilized between dissolution-rate studies and a physiological test in rabbits depending upon the drug's presence *in vivo* at its site of action. This paper reports the results of these studies for tablets prepared by varying the manner in which the drug was added to the tablet formulations.

EXPERIMENTAL

Test Products—Three different formulations were studied initially. Formulation A was prepared by incorporating anagestone acetate into the tablet formulation as a solution in methylene chloride. Formulation B was prepared by adding micronized anagestone acetate to the tablet formulation in the dry form. Formulation C was prepared by incorporating anagestone acetate powder (30 mesh) into the tablet formulation in the dry form.

Two additional test formulations, D and E, were prepared in the same manner as that described for Formulation A. Formulation D was identical to Formulation A in all respects. Formulation E differed from Formulation D in that it contained 10% starch, whereas Formulation D contained 22% starch.

All the tablet formulations contained the same amount of anagestone acetate (2 mg.), and each tablet weighed 100 mg.

The weight, hardness, and thickness of these formulations were essentially the same, and the disintegration times (with disks) ranged from 4 to 6 min. for all five formulations.

Dissolution Apparatus—An assembly similar to that described by Levy and Hayes (3) was used in the dissolution-rate studies. The apparatus was modified slightly by placing a coarse screen[2] 2.54 cm. (1 in.) above the bottom of the 400-ml. Griffen beaker. A four-blade 5-cm. diameter metal stirrer, attached to a stirring motor affording precise speed control, was used. Due to the very low aqueous solubility of anagestone acetate, a dissolution medium of 25% *tert*-butanol in water was employed to improve the "sink" conditions of the system. Three hundred milliliters of this dissolution medium was placed in the beaker, and the system was equilibrated at 37°. The stirrer was immersed in the dissolution medium to

[1] Anagestone acetate, a progestational agent, is 6α-methyl-4-pregnen-17-ol-20-one acetate, supplied by the Organic Chemistry Division of Ortho Research Foundation.

[2] Ten mesh, No. 23 (0.025 in.) W & N gauge woven stainless steel.