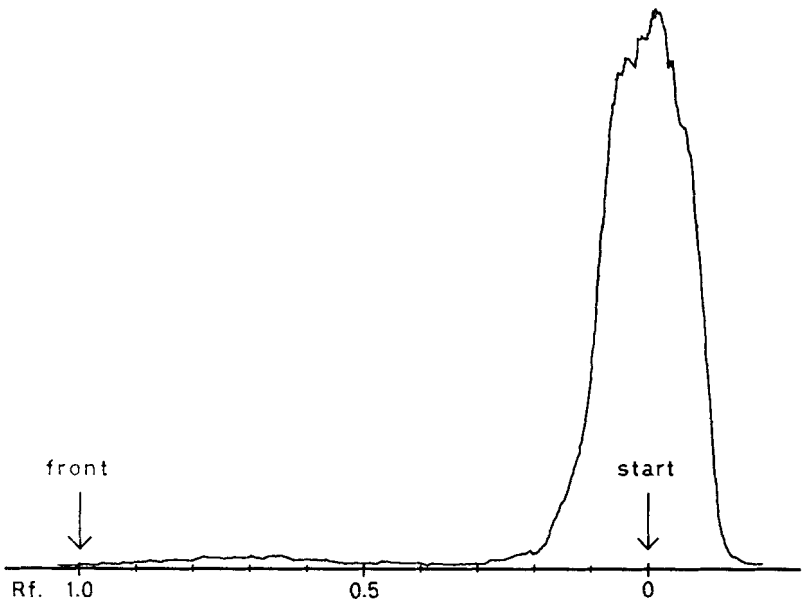

FIG. 3. Radio-paper-chromatogram of ^{131}I-labelled elastase.

Paper-chromatography : Toyo roshi No. 51; solvent, 95 % ethanol : 2 N ammonia (9 : 1).

Radio-scanning : speed, 12.5 mm/min; time const., 1 sec; slit-width, 1.5 mm with a windowless gas-flow counting system (Aloka paper chromatogram scanner model TRM-1B).

REFERENCES

1. HALL, D. A. — « Elastolysis and Ageing », Charles C. Thomas Publisher, Illinois, 1964.
2. BALO, J. — *Intern. Review Connective Tissue Res.*, **1** : 241 (1963).
3. STEINBERG, D., VAUGHAN, M., ANFINSEN, C. B. and GORRY, J. — *Science*, **126** : 447(1957).
4. GREENWOOD, F. C. and HUNTER, W. M — *Biochem. J.*, **89** : 114 (1963).
5. The enzymatic activity was determined according to Sachar's method. SACHAR, L. A., WINTER, K.K., SICHER, N. and FRANKEL, S. *Proc. Soc. Exp. Biol. Med.*, **90**: 323 (1955).
6. LOEVEN,W. A. — *Acta Physiol. Pharmacol. Neerl.*, **12** : 57 (1963).
7. ^{131}I-sodium iodide was obtained from the Daiichi Radioisotope Laboratory.

Synthesis of 7-chloro-1-[cyclopropylmethyl-(methylene-^{14}C)]-5-phenyl-1H-1,4-benzodiazepin-2 (3H)-one and 7-chloro-1-(cyclopropylmethyl)-5-phenyl-1H-1,4-benzodiazepin-2 (3H)-one-5-^{14}C

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In order to more easily study the absorption, excretion, and distribution patterns of 7-chloro-1-(cyclopropylmethyl)-5-phenyl-1H-1,4-benzodiazepin-

2(3H)-one (I) [1] in various biological species, and to investigate the metabolites [2], the new drug was labeled with ^{14}C, both in the methylene carbon of the cyclopropylmethyl group, (I*a*), and in the carbon at position 5 of the benzodiazepine ring (I*b*).

The synthetic route used to prepare both I*a* and I*b* is shown in Figure 1 [3]. The ^{14}C was introduced into I*a* using cyclopropylcarboxylic-(carbonyl-^{14}C) acid (II*a*). The subsequent radioactive intermediates will be related to the synthesis of I*a* by the use of the appropriate Roman numeral followed by an "*a*".

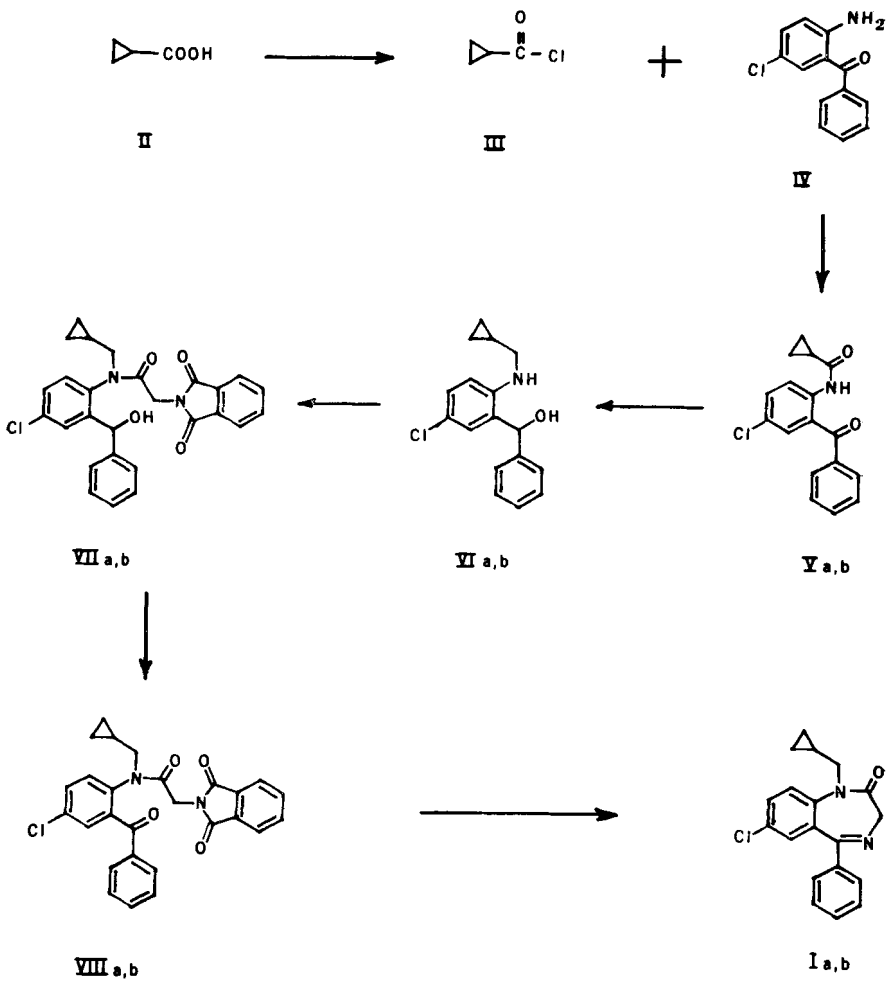

FIG. 1. Synthesis of
7-Chloro-1-cyclopropylmethyl-5-phenyl-1H-1,4-benzodiazepin-2(3H)-one-^{14}C.

When 2-amino-5-chlorobenzophenone-(carbonyl-^{14}C) (IV*b*) was used, the ^{14}C was incorporated into the ring system as I*b*. Similarly, the subsequent labeled intermediates will be linked to I*b* by the use of the appropriate Roman numeral followed by a "*b*".

The II*a* was prepared in 51 % over-all yield via published procedures [4-6] from potassium cyanide-^{14}C and 1-bromo-3-chloropropane. The acid chloride (III*a*) was prepared *in situ* and was allowed to react with IV. The crude 2-cyclopropyl-carboxamido-(carboxyl-^{14}C)-5-chlorobenzophenone was reduced with lithium aluminium hydride to give 2-cyclopropylmethylamino-(methylene-^{14}C)-5-chlorobenzhydrol (VI*a*) in an over-all yield of 45 % from II*a*. When phthalimidoacetyl chloride was allowed to react with VI*a*, 2[N-phthalimidoacetyl-N-cyclopropylmethyl-(methylene-^{14}C)] amino-5-chlorobenzhydrol (VII*a*) was produced in 62 % yield. Oxidation of VII*a* with chromium trioxide gave 2-[N-phthalimidoacetyl-N-cyclopropylmethyl-(methylene-^{14}C)]amino-5-chlorobenzophenone (VIII*a*) in quantitative yield. When VIII*a* was allowed to react with hydrazine hydrate, I*a* was obtained in 69 % yield. The over-all chemical and radiochemical yield for I*a* was 8.8 % from the potassium cyanide-^{14}C.

Benzoyl-(carboxyl-^{14}C) chloride was prepared from benzoic-(carboxyl-^{14}C) acid and was allowed to react with *p*-chloroaniline in a 2.2 to 1 molar ratio using zinc chloride as catalyst. Using the procedure described by Dziewoński and Sternbach [16], the crude product was hydrolyzed with sulfuric acid and after purification, gave IV*b* in 44 % yield. In addition, 51 % of the benzoic-(carboxyl-^{14}C) acid was recovered.

The IV*b* was acylated with III, and the crude product, when reduced with lithium aluminum hydride, gave a 58 % yield of VI*b*. This was allowed to react with phthalimidoacetyl chloride and gave a near quantitative yield of VII*b*. Oxidation of VII*b* with chromium trioxide gave a 90 % yield of VIII*b*. When VIII*b* was allowed to react with hydrazine hydrate, I*b* was obtained in a 64 % yield. The chemical conversion for I*b* was 14.7 % while the radiochemical conversion was 12.3 % from benzoic-(carboxyl-^{14}C) acid.

The physical constants determined for both I*a* and I*b* are contained in the Experimental.

Experimental.

*Cyclopropylcarboxylic-(carboxyl-^{14}C) acid (II*a*).*

Crude 4-chlorobutyronitrile-1-^{14}C from 977 mg (15 mM) potassium cyanide-^{14}C [5 mCi [7]] and 3.15 g (20.1 mM) 1-bromo-3-chloropropane was cyclized and hydrolyzed using literature procedures [4-6] to give 660 mg (51 %) of II*a* as a colorless oil which boiled at 94-96° C/26 mm.

*2-[Cyclopropylcarboxamido-(carboxyl-^{14}C)]-5-chlorobenzophenone (V*a*).*

To a solution of 660 mg (7.7 mM) II*a* and 1.78 g (7.7 mM) 2-amino-5-chlorobenzophenone [15] in 19.4 ml dry benzene was added a solution of

0.56 ml (7.7 mM) thionyl chloride in 5 ml dry benzene dropwise over 30 min. The solution was allowed to reflux for 2 hrs., cooled, and basified with sufficient 10 % aqueous NaOH to adjust the pH to 8. The organic phase was washed with water until neutral, dried with magnesium sulfate, filtered, and the solvent removed. The 2.31 g of crude oil was used directly to prepare VI*a*.

2-[Cyclopropylmethyl-(methylene-^{14}C)]-5-chlorobenzhydrol (VIa).

To a magnetically stirred suspension of 394 mg (10.4 mM) of lithium aluminum hydride (LAH) in 10 ml dry tetrahydrofuran cooled in an icebath, was added a solution of 2.31 g (7.7 mM) V*a* in 5.4 ml dry tetrahydrofuran over 40 minutes. The suspension was allowed to reflux for 5 hrs., cooled, and 1.03 ml of water added cautiously with stirring. The solid was filtered and extracted with five 5 ml portions of tetrahydrofuran. The combined filtrates were dried with magnesium sulfate, filtered, and the solvent removed. The residue was extracted with three 10 ml portions of hot skellysolve C [8]. The insoluble oil was discarded. The skellysolve C extracts were combined and the solvent removed. This residue was dissolved in 4 ml benzene and allowed to percolate through a 10 g column of silica gel [9]. Four 5 ml portions of the eluate were collected, combined, and the solvent removed. The residue was recrystallized from 8 ml skellysolve C to give 1.01 g (45 %) of VI*a* which melted at 82-83° C.

*2-[N-Phthalimidoacetyl-N-cyclopropylmethyl-(methylene-^{14}C)amino-5-chlorobenzhydrol (VII*a*).*

To a cooled, magnetically stirred suspension of 1.00 g (3.47 mM) of VI*a* and 191 mg (4.72 mM) sodium hydroxide in 0.64 ml water and 1.5 ml toluene was added in four equal portions every 5 min., a solution of 965 mg (4.30 mM) phthalimidoacetyl chloride [18] in 3.5 ml dry toluene. The transfer was completed with two 0.2 ml portions of dry toluene. After stirring at room temperature for 1.5 hrs., the suspension was filtered and the solid recrystallized from 2.86 ml hot acetonitrile. The organic phase of the filtrate was dried with magnesium sulfate, filtered, and the solvent removed. The oily residue was recrystallized from 1 ml hot acetonitrile. The recrystallized solids were combined to give 1.01 g (62 %) of VII*a* which melted at 141-144° C.

*2-[N-Phthalimidoacetyl-N-cyclopropylmethyl-(methylene-^{14}C)]amino-5-chlorobenzophenone (VIII*a*).*

To a magnetically stirred solution of 234 mg (2.34 mM) chromium trioxide in 0.054 ml water and 2.68 ml glacial acetic acid was added 1.01 g (2.12 mM) finely ground VII*a* at room temperature gradually over 30 minutes. The suspension was heated to 80° C for 1 hr. To the hot solution was added 6.35 ml water and the oil, which precipitated rapidly, solidified. This solid was ground in a mortar with pestle in the presence of 3 ml water. The solid was filtered to give 1.00 g (100 %) of VIII*a* which melted at 155-159° C.

7-Chloro-1-[cyclopropylmethyl-(methylene-^{14}C)]-5-phenyl-1H-1,4-benzodiazepin-2(3H)-one (Ia).

A suspension of 1.00 g (2.11 mM) VIII*a* and 0.119 ml (2.32 mM) hydrazine hydrate in 5.2 ml methanol was allowed to reflux for 5 hrs. The hot suspension was filtered to remove the insoluble 1,4-phthalazinedione. The solvent was removed from the filtrate, and the yellow residue was extracted with five 5 ml portions of chloroform. The combined organic phases were washed with five 5ml portions of 12N ammonium hydroxide. The aqueous washes were combined and reextracted with two 5 ml portions of chloroform. The combined organic phases were dried with magnesium sulfate, filtered, and the solvent removed. The resulting oil was dissolved in 1 ml ethanol and filtered through a bed of hyflo supercel [10]. The adsorbent was washed with three 0.3 ml portions of ethanol, and the combined filtrates were concentrated to 1 ml by allowing a stream of nitrogen to blow over the warmed solution. Two portions of 1.25 ml each of skellysolve C were added and the resulting precipitate was filtered to give 471 mg (69 %) of I*a* which melted at 144-145° C.

The specific activity was 0.93 mCi/g by liquid scintillation counting [11]. A thin-layer chromatogram (TLC), using a plate coated with a 250 micron layer of silica gel G (Analtech, Inc.), when irrigated with a mixture of 3 parts benzene and 1 part ethyl acetate, gave a single radioactive spot [12] at an R_f of about 0.4, which was identical with an authentic sample of I when visualized with iodine vapors. An infrared spectrum determined as a mull exhibited maxima and minima only at the same wavelengths as an authentic sample [16].

2-Amino-5-chlorobenzophenone-(carbonyl-^{14}C) (IVb).

Using the procedure of Heidelberger and Rieke [13], 15.26 g (125 mM) benzoic-(carboxyl-^{14}C) acid (55 mCi, specific activity 0.44 mCi/mM) [14] were converted into 17.5 g (100 %) of benzoyl-(carboxyl-^{14}C) chloride, bp = 75-79° C/16 mm. One-half of this acid chloride was added dropwise over 1 hr. to a magnetically stirred melt of 7.28 g (56.8 mM) *p*-chloroaniline followed by 7.73 g (56.8 mM) zinc chloride (dried in vacuo at 100° C for 1 hr.). The temperature was raised to 180° C and the remaining acid chloride added over 1 hr. The temperature was increased rapidly to 220° C and maintained at this temperature for 1 hr. The melt was poured over 107 g ice containing 7 ml concentrated hydrochloric acid. The suspension was filtered after stirring overnight, and the solids were washed with water. This mixture was allowed to reflux for 4 hrs. in 87 ml of a mixture of 22.2 parts concentrated sulfuric acid, 14.2 parts water, and 4.35 ml absolute ethanol. The reaction was poured over 260 g ice and extracted with three 200 ml portions of diethyl ether. The organic phases were combined and washed with five 60 ml portions of 1N ammonium hydroxide. The aqueous washes were combined and put aside. The organic phase was dried with magnesium sulfate, filtered, and the solvent removed. The residue was dissolved in 200 ml hot skellysolve C and then

concentrated to 60 ml. After cooling, the precipitate was filtered to give 5.66 g (43.5 %) of IV*b*, which melted at 94-95° C. Chattaway [15] has reported a melting point of 100° C for this compound. The specific activity [11] was 0.44 mCi/mM. A TLC in the system used to characterize I*a* gave a single radioactive spot at an R_f of about 0.7 which was identical with an authentic sample of IV when visualized after exposure to idone vapors.

The dilute ammonium hydroxide washes from above were acidified with sulfuric acid to a pH of 2, and the precipitate filtered. The filtrate was extracted with three 25 ml portions of diethyl ether. The organic phases were combined, dried with magnesium sulfate, filtered, and the solvent removed. Both solids were combined and sublimed in vacuo at 80-90° C to give 7.75 g (50.6 % recovery) of benzoic-(carboxyl-^{14}C) acid, which melted at 121-2° C. The specific activity [11] was 0.43 mCi/mM. A TLC on glass plates coated with silica gel GF (Analtech, Inc.), when irrigated with a mixture of 100 parts ethanol, 12 parts water, and 16 parts 8N ammonium hydroxide, gave one spot at an R_f of about 0.8 which was identical with an authentic sample of benzoic acid when visualized under UV light, and contained 99 % of the radioactivity on the plate.

2-Cyclopropylcarboxamido-5-chlorobenzophenone-(carbonyl-^{14}C) (Vb).

The 5.66 g of IV*b* were converted into crude V*b* by the same method described for V*a*, which was used directly to prepare VI*b*.

2-Cyclopropylmethylamino-5-chlorobenzhydrol-α-^{14}C (VIb).

LAH reduction of the crude V*b* by the procedure described for VI*a* gave 4.0 g (57.7 %) of VI*b*, which melted at 83-84° C.

2-(N-Phthalimidoacetyl-N-cyclopropylmethyl)amino-5-chlorobenzhydrol-α-^{14}C (VIIb).

The 4.0 g of VI*b* were used to prepare VII*b* by the method described for VII*a*. Filtration of the solid gave 6.6 g (102 %) of VII*b* which, without further purification, melted at 144-145° C.

2-(N-Phthalimidoacetyl-N-cyclopropylmethyl)amino-5-chlorobenzophenone-(carbonyl-^{14}C) (VIIIb).

The procedure described for the preparation of VIII*a* was used to convert the 6.6 g of VII*b* into 5.8 g (89.9 %) of VIII*b*, which melted at 155-159° C.

7-Chloro-1-cyclopropylmethylamino-5-phenyl-1H-1,4-benzodiazepin-2(3H)-one-5-^{14}C (Ib).

The 5.8 g of VIII*b* were used to prepare I*b* by the method described for I*a*. A yield of 2.55 g (64 %) was obtained which melted at 144-145° C.

The specific activity was 1.31 mCi/g (0.43 mCi/mM) [11]. A TLC using the system described in I*a* gave a single radioactive spot at an R_f of about

0.4 which was identical with an authentic sample, when visualized after exposure to iodine vapors. An infrared spectrum determined as a mull exhibited maxima and minima only at the same wavelengths as an authentic sample. The molecular extinction coefficient measured in ethanol at 228 nm was 31,400, which is 101 % of an authentic sample.

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Edward J. MERRILL and Gerald G. VERNICE

Department of Analytical/Physical Chemistry
Warner-Lambert Research Institute, Morris Plains, N. J. 07950.

REFERENCES

1. The accepted generic name for this compound is prazepam.
2. DiCARLO, F. J., CREW, M. C., MELGAR, M. D. and HAYNES, L. J. — *J. Pharm. Sci.*, **58** : 960 (1969).
3. McMILLAN, F. H. and PATTISON, I. — U. S. 3,192,199 (6/29/65).
4. BORSOOK, H., DEASY, C. L., HAAGEN-SMIT, A. J., KEIGHLEY, G., and LAWY, P. H. — « Org. Synth. with Isotopes », Pt. 1, A. Murray and D. L. Williams, Eds., Interscience Publishers, Inc., N. Y., 1958, p. 451.
5. SCHLATTER, M. J. — *J. Amer. Chem. Soc.*, **63** : 1733 (1941).
6. McCLOSKEY, C. M. and COLEMAN, G. H. — Org. Synth. Coll., Vol. 3, 1955, p. 221.
7. Purchased from New England Nuclear Corp., Boston, Mass.
8. Skellysolve C is principally *n*-heptane and is available from Skelly Oil Co., El Dorado, Kansas.
9. Silica gel of 100-200 mesh is an adsorbent manufactured by Grace Chemical Co., and is available from Fisher Scientific Co., Fairlawn, N. J.
10. Hyflow supercel is a filter aid manufactured and sold by Johns-Manville Celite Division, New York, N. Y.
11. Packard Model 314X liquid scintillation spectrometer was used. The efficiency was determined by the channels ratio technique in a cocktail composed of 7.0 g PPO (2,5-diphenyloxazole), 0.3 g dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene] and 100 g naphthalene in 1 l 1,4-dioxane. (See F. N. Hayes, Packard Technical Bulletin #1, 1963, p. 4).
12. A Packard Model 7200 radiochromatogram scanner was used
13. HEIDELBERGER, C. and RIEKE, H. S. — *In* « Organic Syntheses with Isotopes », Pt. 1, A. Murray and D. L. Williams, Eds., Interscience Publishers, Inc., N. Y. C., 1958, p. 379.
14. Purchased from International Chemical and Nuclear Corp., Irvine, California 92664.
15. CHATTAWAY, F. D. — *J. Chem. Soc.*, **85** : 340 (1904).
16. DZIEWOŃSKI, K. and STERNBACH, L. — *Chem. Abstr.*, **30** : 2971 (1936).
17. The authentic sample was supplied by A. D. Lewis and M. Goodenough of these laboratories.
18. GABRIEL, S. — *Ber.*, **40** : 2649 (1907).