(6) E. F. Godefroi, H. J. J. Loozen, and J. Th. J. Luderer-Platje, *Recl. Trav. Chim. Pays-Bas*, **91**, 1383 (1972).
(7) B. Tertov, U. V. Burykin, and I. D. Sadekov, *Khim. Geterotsikl. Soedin.*, 520 (1969); *Chem. Abstr.*, **71**, 124328y (1969).
(8) L. A. M. Bastiaansen and H. M. J. M. van As, unpublished results. For a related but independent study of this reaction, see D. S. Noyce, G. T. Stowe, and W. Wong, *J. Org. Chem.*, **39**, 2301 (1974).

Preparation of [^{18}F]Haloperidol

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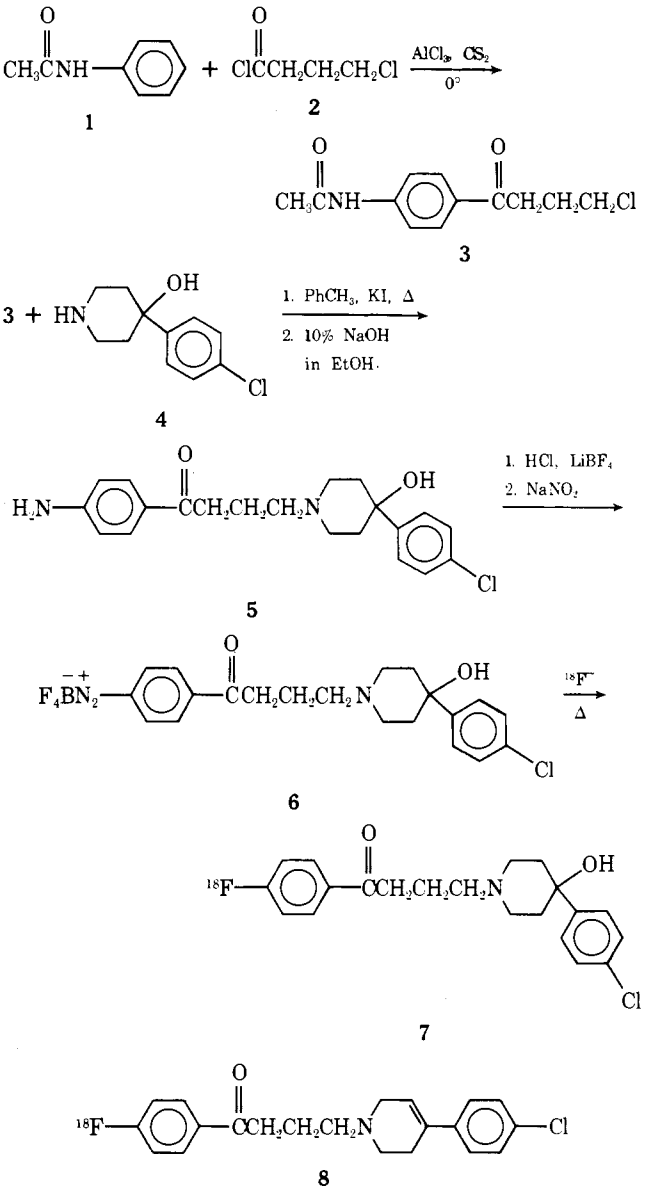
A procedure is described that permits the preparation of [^{18}F]haloperidol in 140 min at specific activities ranging from 4–5 μCi mg^{-1}. A key step in the synthetic route involves the incorporation of ^{18}F into the molecule through a Schiemann-type reaction, which involves the pyrolysis of the diazonium tetrafluoroborate salt of 4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4′-aminobutyrophenone.

In vivo tissue distribution studies with ^{14}C-labeled compounds require serial sacrifice of essentially identical isogenic animals followed by laborious analytical assessment of changes in ^{14}C activity in organs as a function of time after administration. The data obtained from such studies are not only inapplicable to man but also are of limited use in any large mammal where colonies of identical isogenic subjects are unavailable.[2] Human studies with compounds labeled with "short-lived," γ-emitting radionuclides can produce much valuable diagnostic information, such as the organ visualization and function studies of nuclear medicine. Such studies also provide the opportunity to perform tissue distribution determinations and pharmacokinetics in man in a nondestructive manner.

One of the more useful radionuclides for bone scanning is ^{18}F which is now in routine use in many medical centers, including our own, in the form of the simple inorganic fluoride.[3a] ^{18}F is a pure positron emitter (0.635 MeV, β+) and the resulting 511 keV annihilation radiation is easily detected with conventional nuclear medicine instrumentation. Its short physical half-life (110 min) results in a low radiation dose to the patient. ^{18}F-Labeled amino acids such as 4-fluorophenylalanine[3,4] and 6-fluorotryptophan[5] have been prepared and evaluated as specific pancreas scanning agents.[4,6] Recently a method for the preparation of fluorine-18 labeled metal fluorides and organic diazonium tetrafluoroborates has been described.[7] This method has been applied in the synthesis of ^{18}F-labeled amino acids. The synthesis of [^{18}F]-5-fluorouracil[8] and [^{18}F]-5-fluoro-Dopa[9,10] has also been reported. The former has been prepared as a potential tumor localizing agent[8] and the latter as a brain scanner.[9] It is interesting to note that a significant loading dose effect on the localization of both [^{18}F]-4-fluorophenylalanine and [^{18}F]-6-fluorotryptophan has been observed in animals.[11,12] Thus, the pancreas to liver ratio increases with decreasing amounts of ^{18}F-labeled amino acids administered. These observations strongly suggest the necessity for development of synthetic methods which can result in carrier-free products of very high specific activity. It is hoped that the availability of such radiopharmaceuticals would permit studies dealing with organ localization of these agents as a function of their loading dose.

Our interest in obtaining data on tissue distribution and pharmacokinetics of the neuroleptic drug haloperidol (**7**) (the chemistry,[13] pharmacology,[14] distribution, excretion, and metabolism in rats[15] of the drug have been studied) by external scintigraphic techniques has led us to devise a synthetic route (Scheme I) for its rapid preparation in the ^{18}F isotopically labeled form. According to this sequence the incorporation of ^{18}F into the molecule occurs at the last step (**6** to **7**) through a Schiemann-type reaction which involves the pyrolysis of the diazonium tetrafluoroborate salt **6**.

Scheme I



The production of haloperidol (7) was favored (37.8% yield from 5) when the pyrolysis of salt 6 was conducted at 145° for 4 min in a xylene–dioxane[9] (3:1) mixture. Under such conditions olefin 8 was found to be the minor product of the reaction. In contrast, however, attempts to run the pyrolysis of 6 for longer periods of time (30 min), or in the absence of a solvent, favored the production of the olefin 8.

The procedure described below permits the preparation of [^{18}F]haloperidol in about 140 min (ca. 1.5 half-lives of the radionuclide) at specific activities ranging from 4 to 5 μCi/mg.

Experimental Section

Melting points were determined on a Fisher-Johns (hot stage) apparatus and are uncorrected. Where analyses are indicated by the symbols of the elements, analytical values were within ±0.3% of the calculated values. Compounds gave satisfactory uv, ir, NMR, and mass spectral data obtained respectively on Cary 15, Beckman IR-8, Varian Associates A-60A, and Hitachi RMU-7 mass spectrometers. Chemical shifts are downfield from the standard Me_4Si. For TLC, precoated silica gel plates (Quanta-Gram, Quantum Industries, Fairfield, N.J.) were used. Compounds 7 and 8 were detected by first spraying the plates with a fluorescein solution[16] followed by visualization under a long-wave ultraviolet lamp. The radionuclidic purity of the ^{18}F was assured by γ spectrometry (Packard 4096 channel analyzer, Model 45) with a Ge(Li) detector (Nuclear Diodes, Inc.). The absence of long-lived β-emitters was shown by liquid scintillation counting (Packard Model 3310) after the complete decay of ^{18}F.

4′-(4-Chlorobutyryl)acetanilide (3).[17] To a mixture of acetanilide (1, 10 g, 0.07 mol), $AlCl_3$ (40 g), and 40 ml of CS_2, a solution of 4-chlorobutyroyl chloride (2, 30 g, 0.21 mol) in 30 ml of CS_2 was added dropwise to 0°. The reaction mixture was then heated at 50° for 2 hr and the supernatant layer of CS_2 discarded. The oily residue was then poured over a mixture of ice–water and the amorphous precipitate filtered and recrystallized from 95% EtOH to give 9.3 g (0.038 mol, 57% yield) of 3: mp 148–150° (lit. mp 147–152°,[17a] 157–159°,[17b] 162–164°[17c]); *m/e* 239 (M^+).

4-[4-(*p*-Chlorophenyl)-4-hydroxypiperidino]-4′-aminobutyrophenone (5). Product 3 (4.80 g, 0.02 mol) was heated in a sealed tube with 9.0 g (0.04 mol) of 4-(*p*-chlorophenyl)-4-hydroxypiperidine (4, mp 145°, Aldrich, Milwaukee, Wis.), 0.05 g of KI, and 75 ml of distilled toluene at 110–115° for 48 hr. Upon cooling the solid phase of the reaction was separated and washed successively with H_2O, $CHCl_3$, and Et_2O. To an ethanolic (95% EtOH) mixture of the product, which was refluxed for 1 hr, 75 ml of 10% NaOH was added and the reflux continued for 40 min. Upon cooling and the addition of 100 ml of H_2O, 5.47 g (0.014 mol, 70% yield from 3) of 5 was obtained. The buff crystals were recrystallized from 95% EtOH: mp 175°; *m/e* 372 (M^+). Anal. ($C_{21}H_{25}ClN_2O_2$) C, H, Cl, N.

Synthesis of Haloperidol (7). To 1 g (2.6 mmol) of 5, 1 ml of concentrated HCl and 7 ml of H_2O were added and the mixture was cooled to 5°. $LiBF_4$ (1.2 g) in 2.4 ml of H_2O, cooled to 5°, was then added dropwise. To this 3 ml of aqueous 10% $NaNO_2$ was added slowly and stirred vigorously for 15 min. The aqueous portion of the reaction mixture was decanted and the brown solid (presumably the diazonium fluoroborate salt 6) washed with cold water (5°), followed by 5 ml of aqueous 5% $LiBF_4$ (5°), and freeze-dried. The dried salt 6 was subsequently pyrolyzed in a mixture of xylene–dioxane[9] (3:1) at 145° for 4 min. The dark brown pyrolysate was then extracted with three 4-ml portions of $CHCl_3$. The xylene–dioxane mixture and the extracts were combined and evaporated to dryness under N_2 to yield 0.49 g of a light yellow residue. When a sample from this residue was examined by two dimensional TLC [silica gel; solvent 1, $CHCl_3$–MeOH–concentrated NH_4OH (96:4:1); solvent 2, 2-propanol–4 *N* NH_4OH (80:20)] it exhibited a major spot with identical R_f values (0.6 and 0.82, respectively) with that of authentic haloperidol. A minor spot exhibiting R_f values (0.75 and 0.95, respectively) identical with those of olefin 8 was also detected. The residue was then chromatographed on 21 g of neutral alumina (Fisher, activity I). Five-milliliter fractions were collected. Elution with $CHCl_3$ gave 0.10 g (11% yield from 5) of a white crystalline product which was shown, after one recrystallization from 95% EtOH, by mixture melting point (mp 92°) and mass spectrometry to be identical with a sample of olefin 8, synthesized as described below. Elution with 2% MeOH in $CHCl_3$ yielded 0.37 g (37.8% yield from 5) of haloperidol (7), the identity of which was unequivocally proven by mixture melting point (mp 149°) and comparative spectroscopic determinations (ir, uv, and mass spectra) with authentic haloperidol.

Preparation of [^{18}F]Haloperidol (7). ^{18}F was produced by the ^{20}Ne(d,α)^{18}F nuclear reaction[18] at the end of which the glass target insert was washed with 7.5 ml of distilled H_2O. The volume of the H_2O was reduced to about 1 ml by heating at 110° and by blowing N_2 into the solution. To the aqueous solution, containing 11.34 mCi of ^{18}F, diazonium salt 6 (prepared from 1 g of 5 as described above) and 2.0 ml of acetone[3] were added. After standing for 5 min (to enhance the incorporation of ^{18}F into the tetrafluoroborate moiety[3] of 6) the mixture was dried by heating at 50° under N_2 and by subjecting it to high vacuum for 5 min. The dried residue was subsequently pyrolyzed in xylene–dioxane (3:1) as described above. The pyrolysis product was chromatographed on neutral alumina first with $CHCl_3$ and subsequently with 2% MeOH in $CHCl_3$ as eluent. The white crystalline residue, obtained from the evaporation of 2% MeOH–$CHCl_3$ eluent fractions, was washed with 2 × 2 ml of Et_2O to yield 0.37 g (1.76 mCi) of [^{18}F]haloperidol. The identity of the product was substantiated by two-dimensional TLC (see details above). A radioscan of the thin-layer plate showed all the activity coincident with the spot of authentic haloperidol. The percent yield of incorporation of ^{18}F into the final product 7 was calculated to be 35.5%. The present method permits the production of [^{18}F]haloperidol at specific activities of about 4–5 μCi/mg in about 140 min.

4-[4-(*p*-Chlorophenyl)-2,5,6-trihydropyridino]-4′-fluorobutyrophenone (8). 5 (1.1 g, 2.8 mmol) was converted to its diazonium salt 6 as described under the preparation of haloperidol. Salt 6 was then pyrolyzed in a mixture of xylene–dioxane (3:1)[9] at 150° for 30 min. The xylene–dioxane mixture was then separated from the resulting brown residue. The latter was extracted with three 15-ml portions of $CHCl_3$, and the extracts were combined with the xylene–dioxane mixture and evaporated to dryness. (Only trace amounts of haloperidol could be detected when a small sample from the residue was examined by TLC, as described above.) The residue was subsequently chromatographed on 10 g of neutral alumina (Fisher, activity I). Elution with $CHCl_3$ gave 0.54 g (1.5 mmol, 50.5% yield) of olefin 8 (*m/e* 357) as a white crystalline product. Recrystallization from 95% EtOH yielded the pure product, mp 95°. Anal. ($C_{21}H_{21}ClFNO$) C, H, N. The NMR of the olefin exhibited a peak at δ 6.05 assigned to the olefinic proton.

References and Notes

(1) Address correspondence to this author at the College of Pharmacy, University of Kentucky.
(2) M. B. Winstead, J. F. Lamb, and H. S. Winchell, *J. Nucl. Med.*, **14**, 747 (1973).
(3) (a) R. W. Goulding and A. J. Palmer, *Int. J. Appl. Radiat. Isot.*, **23**, 133 (1973), and references cited therein; (b) R. M. Hoyte, S. S. Lin, D. R. Christman, H. L. Atkins, W. Hauser, and A. P. Wolf, *J. Nucl. Med.*, **12**, 280 (1971).
(4) M. F. Cottrall, D. M. Taylor, and T. J. McElwain, *Br. J. Radiol.*, **46**, 277 (1973).
(5) R. M. Hoyte, S. S. Lin, H. L. Atkins, D. R. Christman, W. Hauser, J. F. Klopper, and A. P. Wolf, *J. Nucl. Med.*, **12**, 367 (1971).
(6) D. M. Taylor and M. F. Cottrall, "New Developments in Radiopharmaceuticals and Labeled Compounds", Copenhagen, 1973, IAEA/SM-171.
(7) J. C. Clark, R. W. Goulding, M. Roman, and A. J. Palmer, *Radiochem. Radioanal. Lett.*, **1412**, 101 (1973).
(8) J. S. Fowler, R. D. Finn, R. M. Lambrecht, and A. P. Wolf, *J. Nucl. Med.*, **14**, 63 (1973).
(9) G. Firnau, C. Nahmias, and S. Garnett, *Int. J. Appl. Radiat. Isot.*, **24**, 182 (1973).
(10) G. Firnau, C. Nahmias, and S. Garnett, *J. Med. Chem.*, **16**, 416 (1973).
(11) 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).
(12) A. P. Wolf, D. R. Christman, J. S. Fowler, and R. M. Lambrecht in ref 6.
(13) P. A. J. Janssen, C. Van de Westeringh, A. H. M. Jageneau, P. J. A. Demoen, B. K. F. Hermans, G. H. P. Van Daele, K. H. L. Schellekens, C. A. M. Van der Eyeken, and C. J. E. Niemegeers, *J. Med. Pharm. Chem.*, **1**, 281 (1959).
(14) P. A. J. Janssen, *Neuro-Psychopharmacol., Proc. Meet. Coll. Int. Neuro-Psychopharmacol., 3rd, 1962*, 331 (1964).

(15) (a) W. Soudijn, I. Van Wijngaarden, and F. Allewijn, *Eur. J. Pharmacol.*, **1**, 47 (1967); (b) G. A. Braun, G. I. Poos, and W. Soudijn, *Eur. J. Pharmacol.*, **1**, 58 (1957).
(16) K. G. Krebs, D. Heusser, and H. Wimmer, "Thin Layer Chromatography", 2nd ed, E. Stahl, Ed., Springer-Verlag, New York, N.Y., 1969, p 878.
(17) (a) P. Adriaan and J. Janssen, British Patent Application 881893 (1961) [*Chem. Abstr.*, **57**, 2198c (1962)]; (b) C. Van de Westeringh, B. Hermans, F. Raeymalkers, and C. Van der Eycken, *Ind. Chim. Belge*, **25**, 1073 (1960) [*Chem. Abstr.*, **55**, 6428g (1961)]; (c) May and Baker Co., British Patent Application 943739 (1959) [*Chem. Abstr.*, **60**, 5522h (1964)].
(18) P. V. Harper, N. Lembares, and H. Krizek, *J. Nucl. Med.*, **12**, 362 (1971).

Analogs of Camptothecin

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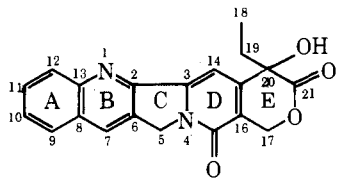
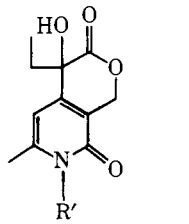
Several compounds having portions of the camptothecin ring system were prepared. These compounds were screened against L1210 lymphoid leukemia with negative results. Two of the analogs which contained the pyridine and hydroxylactone D and E rings were also screened for inhibition of DNA and RNA syntheses in HeLa cells. Each of these analogs had decreased activity as compared with camptothecin and there was no degradation of DNA in the HeLa cells. This suggests that the D and E rings are not a sufficient requirement for camptothecin-like activity.

The early animal tests showed the alkaloid camptothecin (**1**) to be a potent antineoplastic agent;[1] however, the toxic effects and lack of activity against gastrointestinal cancer showed **1** to be ineffective as a chemotherapeutic agent.[2] The effectiveness of camptothecin against leukemia in experimental animals and the unusual effects it has on macromolecular synthesis[3] have caused an interest in determining the structural features of camptothecin associated with this activity.

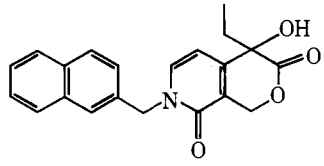


camptothecin (**1**)

Previous studies have indicated that the functional groups of the hydroxylactone ring E must be present to maintain the activity of camptothecin. Thus substitution of the hydroxyl group at C-20 by halogen,[4] acetoxy,[4] ethyl,[5] or hydroxymethyl[6] gives compounds showing reduced or lack of cytotoxicity in animals. Replacement of the hydroxyl group by hydrogen gave a desoxy compound inactive against tumors[4] but showing inhibition of DNA–RNA synthesis.[3] Reduction of the C-21 carbonyl group also caused a loss of activity.[4] The activity of camptothecin is maintained if the lactone ring is opened to the alkali metal salt or amide.[4] These data suggest that the D and E rings of camptothecin are necessary for activity. Only limited information about the biological activity of simple analogs of camptothecin is available; however, the report that **2** has only 0.01 the activity of camptothecin[7] and the weak inhibition of RNA synthesis of **3**[6] suggested that the D and E rings were a necessary but not a sufficient requirement for activity.



2, R = R′ = H
3, R = CH_3; R′ = n-C_4H_9



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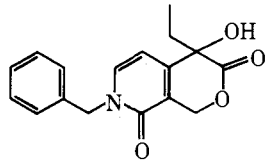

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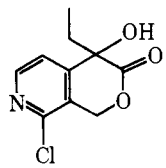

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Results and Discussion

In an attempt to determine the structural requirements required for activity, several series of analogs were prepared, three of which (**4**, **5**, and **6**) contain the D and E ring systems comparable to camptothecin (**1**). The syntheses were described previously.[8] The analog **4** contains the same number of cyclic atoms as camptothecin; however, the nitrogen at position 1 is replaced by carbon and the C-2–3 bond is not closed. This very close analog was inactive in the L1210 leukemia screen (Table I) and was less active than camptothecin as an inhibitor of nucleic acid synthesis in HeLa cells.[9] Replacement of the β-naphthylmethyl group on nitrogen by a benzyl group (**5**) gave a marked decrease in inhibition of macromolecular synthesis. Neither of these analogs caused degradation of DNA, a property possibly related to the suppression of tumor growth,[3] and thus the inactivity in the L1210 screen was expected.

Table I lists additional compounds related in structure to camptothecin (**1**) whose syntheses have been reported.[10] **7** contains the atoms of D and E rings and **8** has all the atoms of camptothecin without closure of the lactone ring. **6** has the D and E ring with the carbonyl group of the pyridone replaced by chlorine.[8] These compounds, as well as the analogs with the B and D rings (type I), the A, B, and D rings (type II), and the B, C, and D rings (type III),[10] were inactive in the L1210 leukemia screen.