Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIX, No. 12

RADIOIODINATION OF 7-METHOXY- AND 6,7-DIMETHOXY-4-BROMOMETHYLCOUMARINS

Janina Baranowska-Kortylewicz, Zbigniew P. Kortylewicz

Department of Radiology (Nuclear Medicine), Harvard Medical School, Shields Warren Radiation Laboratory, 50 Binney Street, Boston, Massachusetts 02115

SUMMARY

Two coumarins, 4-bromomethyl-6,7-dimethoxy-2-oxo-2*H*-benzopyran (1) and 4-bromomethyl-7-methoxy-2-oxo-2*H*-benzopyran (3), were radioiodinated using trifluoroacetyl hypoiodite. The dimethoxy derivative 1 gave only a single regioisomer 3-[^{125}I]iodo-4-bromo-methyl-6,7-dimethoxy-2-oxo-2*H*-benzopyran (2). The average yield for no-carrier-added preparations of 2 was 30%. $^{125/127}$I-2 and ^{127}I-2 were produced in over 50% yield. In no-carrier-added syntheses 7-methoxy-coumarin 3 gave 3-[^{125}I]iodo-4-bromomethyl-7-methoxy-2-oxo-2*H*-benzopyran (5) as the major product (30%) accompanied by a small amount (3-7%) of the 6-[^{125}I]iodo-analog 4. When an equimolar amount of iodide was used (i.e. carrier-added syntheses) 4 and 5 were produced in over 20% yield each. Under these conditions the 8-iodo-regioisomer was not formed from either 1 or 3 possibly as the result of steric effects. Sonication greatly accelerated rates of radioiodination reducing the time required to achieve quantitative substitution of ^{127}I from about 4 h to 20 min.

KEY WORDS: ^{125}I-radioiodination; trifluoroacetyl hypoiodite; 4-bromomethyl-6,7-dimethoxy-2-oxo-2*H*-benzopyran; 4-bromomethyl-7-methoxy-2-oxo-2*H*-benzopyran; coumarin.

INTRODUCTION

Radiolabeled reagents that form conjugates with proteins and can be photochemically activated to undergo further reactions with peptide residues are widely used in the determination of structure-function relationships and in the radiolabeling of proteins.

0362–4803/91/121301–07$05.00

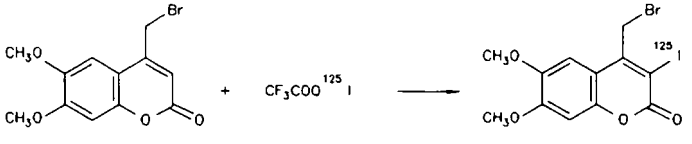
Received 12 July, 1991
Revised 6 September, 1991

The most common of these compounds contain functional groups that can be converted into highly reactive carbenes upon photoactivation (1,2). These react indiscriminately with C=C, NH, OH and CH always resulting in random labeling of proteins. In addition, side-reactions with surrounding solvents reduce the conjugation yield. Derivatives of 2-oxo-2*H*-benzopyran described in this paper react selectively with sulfhydryl groups of proteins. The subsequent photoactivation of coumarin-protein conjugates produces cross-links only when two coumarin residues are located in immediate proximity.

RESULTS AND DISCUSSION

The iodination of substituted 2-oxo-2*H*-benzopyrans (1, 3) was accomplished by the modified Prévost reaction (3-5). Trifluoroacetyl hypoiodite generated *in situ* from I_2 or ICl, the latter obtained by the oxidation of NaI with *N*-chlorosuccinimide or chloramine-T, substituted exclusively the 3-position in the lactone portion of the coumarin ring (1) to give 3-iodo-4-bromomethyl-6,7-dimethoxy-2-oxo-2*H*-benzopyran (2) (Scheme 1). The time required to achieve quantitative substitution was substantially reduced, from about 4 h to 20 min, when the reaction mixture was sonicated.

Scheme 1



1 2

Radioiodination of 1 and 3 was conducted under identical conditions. Sodium [^{125}I]iodide was oxidized in the aqueous medium, then extracted with chloroform, and dried

over magnesium sulfate. Thus prepared radioiodine in the presence of CF_3COOAg gave a single radioactive product **2** with 30% yield in no-carrier-added and over 50% yield in carrier-added syntheses. The radioiodinated **2** was purified on a C_{18} reverse phase column (Fig. 1). The additional radioactive peak observed on an HPLC profile most likely corresponds to $CF_3COO^{125}I$ since it also appears when ^{125}ICl is mixed with silver trifluoroacetate without **1**. Attempts to iodinate **1** in the absence of this silver salt using I_2, ICl and NaI with a variety of oxidants (e.g. iodogen, chloramine-T, *N*-chlorosuccinimide) routinely used in radioiodinations resulted in the recovery of the unchanged starting material. At elevated temperatures in methanol as the reaction medium, only a small amount of 4-methoxymethyl-6,7-dimethoxy-2-oxo-2*H*-benzopyran and traces of 3-[(2-hydroxy-4,5-dimethoxy)phenyl]-4-bromo-2-butenoic acid methyl ester were isolated.

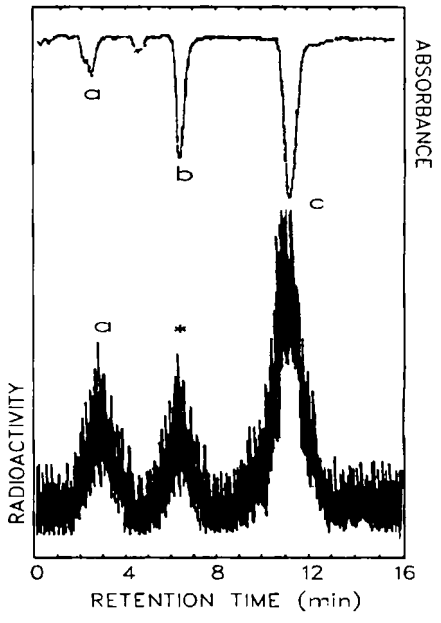


Figure 1. Reverse phase HPLC analysis for iodination of **1**. Upper profile corresponds to nonradioactive reaction with uv detection at 280 nm; lower profile represents $^{125/127}I$-iodination; (a) iodide; (b) unreacted **1**; (c) product **2**; (*) CF_3COOI or 4-iodomethyl-**1**.

Compounds **4** and **5** were obtained in the same way, but at least 30 min of sonication at room temperature was required to give satisfactory iodination yields. The major product obtained was the 3-[^{125}I]iodo-compound **5** accompanied by a small amount (3-7%) of the 6-[^{125}I]-substituted coumarin **4** (Scheme 2). The reaction carried out with an equimolar amount of ^{127}I or a mixture of ^{125}I and ^{127}I produced about 50% of iodinated products with each of the isomers obtained in over 20% yield. The separation of iodinated regioisomers was achieved on an HPLC silica gel column (Fig. 2). There were substantial

Scheme 2

losses of radioactivity (up to 40%) due to the formation of an insoluble material, probably silver [^{125}I]iodide, as indicated by the TLC analysis of the crude reaction mixture (inset in Fig. 2). This could be minimized by maintaining strictly anhydrous conditions and prolonging the oxidation time of iodide.

Under these conditions the formation of 8-iodo-coumarin from either 1 or 3 was not observed. Therefore the substitution at the C8 carbon was attempted on 1 treated first with one molar equivalent of NaOH in tetrahydrofuran followed by CF_3COOI or ICl. It was expected that upon opening of the lactone ring, the additional activating effect of the hydroxy group would direct the iodination to take place predominantly at the C8 position. However after acidification of the reaction mixture only the starting material was recovered. This lack of reactivity at the C8 position of 1 and 3 can most likely be attributed to steric factors.

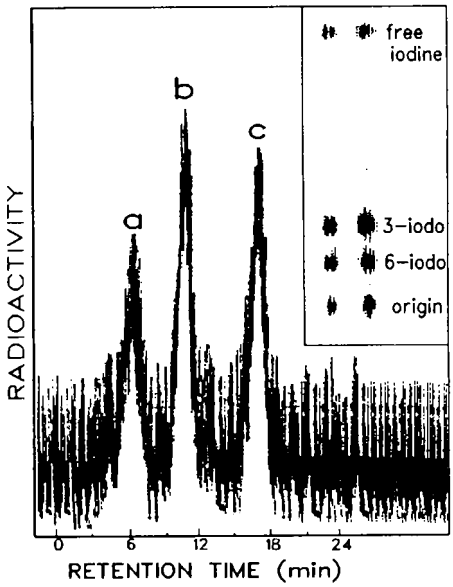


Figure 2. Silica gel HPLC analysis of filtered reaction mixture obtained during [$^{125/127}$I]-iodination of 3; (a) iodine; (b) 3-[$^{125/127}$I]-5; (c) 6-[$^{125/127}$I]-4. Inset: scan of silica gel thin layer radiochromatography plate.

Radiolabeled coumarins 2 and 4 react selectively, like their parent compounds, with free sulfhydryl groups of proteins and their conjugates undergo photocycloaddition to give dimerized proteins (6).

EXPERIMENTAL PROCEDURES

All chemicals were reagent grade. Sodium [^{125}I]iodide (specific activity 2200 Ci/mmol) in 10^{-5} *M* NaOH was purchased from DuPont (Billerica, MA). HPLC analyses were conducted on either a C_{18} reverse phase column using CH_3CN/H_2O (1/1; v/v) as solvent at a flow rate of 1 mL/min or a Maxsil 5 silica column (250 x 4.6 mm or 250 x 10 mm; Phenomenex, Rancho Palos Verdes, CA) with CH_2Cl_2/hexane (80/20; v/v) as solvent at a flow rate of 1 mL/min with detection at 280 nm. The radioactive species were detected with a NaI(Tl) 3-in crystal well detector. TLC was done on silica gel plates ($60F_{254}$) in $CHCl_3/CH_3OH$ (100/1; v/v) or $CHCl_3$. ^{1}HNMR spectra were recorded on a Varian T60 spectrometer. Melting points (mp) were taken on a Fisher-Johns melting point apparatus and are uncorrected. The ultrasonic bath (Ultrasonic Devices-Heat Systems, Inc., Plainview, N.Y.) at an energy of 55 kHz was used for reactions run with sonication. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN).

3-Iodo-4-bromomethyl-6,7-dimethoxy-2-oxo-2*H*-benzopyran (2). All iodination reactions were performed in a vessel shielded from light. To a stirred mixture of 1.0 g (3.34 mmol) 4-bromomethyl-6,7-dimethoxy-2-oxo-2*H*-benzopyran (1) and silver trifluoroacetate (0.74 g, 3.34 mmol) in 75 mL of anhydrous $CHCl_3$ a solution of an equimolar amount of iodine (0.85 g) in 25 mL $CHCl_3$ was added over a 30-min period. This was followed by additional 0.5-mL aliquots of I_2 solution (0.1 g/mL) until no decolorization was observed. The mixture was stirred for 4 h at room temperature or sonicated for 20 min. After the substitution was complete, as indicated by TLC, the precipitated AgI was removed by filtration. The filtrate was washed with 0.01% aqueous $NaHSO_3$ and water, and dried over anhydrous $MgSO_4$. The residue obtained after evaporation of $CHCl_3$ was chromatographed on a flash silica gel column ($CHCl_3/CH_3OH$; 200/1; v/v) to give 1 g (70%) of the title compound as fine yellow crystals (mp 234-

235°C). Anal. Calcd. for $C_{12}H_{10}BrIO_4$: %C 33.90; %H 2.37; %Br 18.80. Found: %C 33.42; %H 2.32; %Br 18.63. ^{1}HNMR ($CDCl_3$/DMSO-d_6): 3.93 (6H, s, 2 x CH_3O); 4.87 (2H, s, CH_2Br); 6.90 (1H, s, C5H); 7.17 (1H, s, C8H). R_f 0.60 in $CHCl_3/CH_3OH$ (100/1); R_T 12 min on a C_{18} reverse phase column.

6-Iodo- (4) and 3-Iodo-4-bromomethyl-7-methoxy-2-oxo-2*H*-benzopyran (5). The reaction was carried out as described for 2 but a 30-min sonication was necessary to effect the iodination. Following flash column chromatography on a silica gel column ($CHCl_3$), 58% of 4 (mp 266-268°C; sub. 220°C) and 14% of 5 (mp 204-205°C; sub. 195°C) were isolated. Anal. Calcd. for $C_{11}H_8BrIO_3$: %C 33.45; %H 2.04; %Br 20.23; %I 32.13. Found: %C 33.51; %H 2.21; %Br 20.63; %I 32.90. ^{1}HNMR ($CDCl_3$/DMSO-d_6) of 4: 3.73 (3H, s, CH_3O); 4.58 (2H, s, CH_2Br); 6.22 (1H, s, C3H); 6.73 (1H, s, C8H); 7.85 (1H, s, C5H). ^{1}HNMR ($CDCl_3$/DMSO-d_6) of 5: 3.90 (3H, s, CH_3O); 4.75 (2H, s, CH_2Br); 6.85 (1H, s, C8H); 6.96 (1H, d, C6H, $J_{5,6}$=4 Hz); 7.58 (1H, d, C5H). R_f 0.27 and 0.48 in $CHCl_3$ for 4 and 5, respectively; R_T on a Maxsil 5, 250 x 10 mm, silica gel column, 30 min for 4 and 23 min for 5, and on 250 x 4.6 mm column, 17 min and 11 min, respectively.

Radioiodination of coumarins. The synthetic procedure was identical for all compounds. In a tightly closed vial a mixture of 1 mg chloramine-T and 0.1 to 1.0 mCi $Na^{125}I$ (specific activity 2200 Ci/mmol for no-carrier-added and 1 Ci/mmol for carrier-added preparations in 10^{-5} *M* NaOH neutralized with equimolar amount of 10^{-5} *M* CH_3COOH) in 0.1 mL of water was stirred for 30 min. Chloroform (0.5 mL) was added to this mixture and the stirring continued an additional 30 min until the majority of the radioactivity was recovered in chloroform. The organic layer was separated, dried over anhydrous $MgSO_4$, and transferred to a vial containing a stirred mixture of 1.0 mg (3.34 μmol) 1 and 1.0 mg (4.0 μmol) CF_3COOAg in 0.1 mL $CHCl_3$. The mixture was allowed

to react at room temperature for 4 h (1) or was sonicated for 30 min (3), and was then filtered to remove AgCl and the unreacted CF_3COOAg. The solvent was evaporated to dryness with nitrogen. The purification of radiolabeled products was achieved using a C_{18} column for 2 and an HPLC silica column for 4 and 5 as described for the derivatives reacted with [^{127}I]iodine. The residue was taken up in 0.4 mL of the appropriate eluant and filtered through a 0.2 μm Millipore filter. After purification on average 30% of the no-carrier-added ^{125}I-2 and ^{125}I-5 and about 50% of the carrier-added $^{125/127}$I-2 and $^{125/127}$I-4,5 (over 20% each) were obtained. The identity of the radiolabeled compounds was verified by comparing their HPLC and TLC behavior with those of the [^{127}I]iodine-substituted derivatives.

ACKNOWLEDGMENTS: This work was made possible by Grant 5 RO1 CA15523 awarded by the National Institutes of Health.

REFERENCES

1. Bayley H. - Laboratory Techniques in Biochemistry and Molecular Biology, vol. 12, Work T.S., Burton R.H., Eds., Elsevier, Amsterdam, 1983.
2. Means G.E. - *Bioconjugate Chem.* 1:2-12 (1990).
3. Wilson C.V. - *Org. React.* 9:332-387 (1957).
4. Chen E.M., Keefer R.M., and Andrews L.J. - *J. Am. Chem. Soc.* 89:428-430 (1967).
5. Barnett J.R., Andrews L.J., and Keefer R.M. - *J. Am. Chem. Soc.* 94:6129-6134 (1972).
6. Baranowska-Kortylewicz J., Adelstein S.J., and Kassis A.I. - *Bioconjugate Chem.*; submitted for publication.