A COMPARATIVE STUDY OF RADIOIODINATION OF SIMPLE AROMATIC COMPOUNDS VIA N-HALOSUCCINIMIDES AND CHLORAMINE-T IN TFAA

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SUMMARY

A comparative study of radioiodination of simple aromatic compounds using N-chlorosuccinimide (NCS), N-bromosuccinimide (NBS), N-chlorotetrafluoro-succinimide (NCTFS) and Chloramine-T (CAT) has been carried out in trifluoroacetic anhydride (TFAA) as an aprotic solvent. Optimization of the iodination procedure has been performed using anisole as the model substrate. A fast and simple "one-pot" synthesis via NCTFS, NCS or CAT leads to iodoanisoles with a radiochemical yield of about 70 %. For comparison, toluene and benzene have also been radioiodinated.

Key words: Iodination, Iodine-123,125,131, N-halosuccinimide, Chloramine-T.

INTRODUCTION

Radiohalogenated compounds are widely used in medical and biochemical research and in medical diagnostic procedures. Among them, radioiodinated compounds have found especially wide application. In the area of nuclear medicine, the number of radioisotopes of iodine used is greater than that of any other element.

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Iodine-123 ($T_{1/2}$ = 13.3 h) was recognised in 1962 as an ideal nuclide for radiopharmaceutical in-vivo applications (1). Its favourable features have been described (2-4). On the other hand, because of its 60 day half-life and small X-ray range, the relatively long-lived iodine-125 is particularly suitable for in-vitro procedures, such as radioimmunoassay.

While a variety of radioiodination methods have been described, most of the techniques reported have limitations, particularly those used at no-carrier-added level (5-15). In general, oxidizing agents which produce electrophilic radiohalogen species are required in aromatic halogenation. We have already demonstrated that N-chloro-tetrafluorosuccinimide (NCTFS) can be used effectively for the radiobromination of aromatic molecules, using carrier-free bromide with trifluoroacetic anhydride (TFAA) as a solvent (16,17). The well-known iodination reagent Chloramine-T (CAT) has also been shown to be very useful for radiobromination in aqueous solutions (18). N-chlorosuccinimide (NCS) can be employed as the oxidizing agent for radiobromination of benzylic positions, e.g. on toluene (19). Recently NCS has been used for the radiobromination of phenolic steroids, i.e. estrogens (20). Both NCS and NBS are suitable for the halogenation of aromatic compounds in both polar protic and polar aprotic solvents (21,22). This paper reports the results of a comparative radioiodination study performed using NCTFS, NCS, NBS, and CAT as oxidizing agents with TFAA as an aprotic solvent.

EXPERIMENTAL

Materials

Analytical reagent grade chemicals were used in this investigation. The benzene derivatives (Merck, Fluka, Schuchardt) were subjected to additional purification by fractional distillation. N-chloro-tetrafluorosuccinimide (NCTFS) was prepared from the corresponding imide by suitable modifications of the method described for the N-bromo-compound (19,23). It was purified by repeated vacuum sublimation and stored in a dry box. Analytical grade N-chlorosuccinimide (NCS) (Fluka AG, Buchs SG Switzerland) was used without further purification. Chloramine-T (N-chloro-4-toluene sulfonamide, sodium salt) was purchased from Merck AG, and N-bromosuccinimide (NBS) from Riedel-de-Haen AG Seelze-Hannover. Iodine-123 was produced at the Jülich Compact Cyclotron CV-28 via the $^{124}Te(p,2n)^{123}I$ reaction (24). Iodine-125 and -131 solutions were purchased from the Radiochemical Centre, Amersham, England.

Methods

Working within a dry box, a weighed amount of N-halosuccinimide or Chloramine-T was introduced into a conical-bottom centrifuge test tube, then 0.5 ml of trifluoroacetic anhydride and a suitable amount of the aromatic substrate were added. Finally 5 µl of a no-carrier-added $Na^{131}I$, $Na^{123}I$ or $Na^{125}I$ solution were introduced by a micro-syringe. The test tube was shielded from light with aluminium foil and kept at room temperature. After the desired reaction time, 1.0 ml of an aqueous solution of 3 % Na_2SO_3 and Na_2CO_3 was cautiously added under ice-cooling to quench the reaction.

1.4 ml chloroform containing 1-2 μg/ml of the expected iodination product as a carrier was then added. After centrifugation the organic layer was separated from the inorganic layer, dried with $CaCl_2$ and aliquots were taken to determine the activity in the organic phase and the total activity.

The analytical method used in the study was similar to those previously described. The reaction mixture containing the iodinated substrate was analysed by discontinuous gas chromatography with a Hewlett-Packard 5750 research chromatograph, trapping the individual peaks on activated charcoal (25). The separation of the iodinated benzene and toluene was performed using a glass column (2 m length, 3.5 mm i.d.) packed with 6 % Bentone-38 and 20 % silicone oil on 60-80 mesh Chromosorb W-AW-DMCS (26,27). The iodinated anisole was separated on a 4-m long glass column (3.5 mm i.d.) filled with 20 % Igepal CO-880 on 60-80 mesh Chromosorb W-AW-DMCS. For quantitative assay, each trap containing a given peak was counted in a well-type Auto-Gamma Scintillation Spectrometer, Model 5375 from Packard. The chlorinated and brominated by-products from anisole were also identified by gas chromatography, using authentic reference standards.

RESULTS AND DISCUSSION

The dependence of the iodination yield on the reaction time is shown in Fig. 1. The results are very similar to those obtained for bromination (16) and indicate that the reaction promoted by NCS is completed after about half an hour. When using NCTFS, about 3 hours are needed. This contrasts with the case of Chloramine-T, for which only a few seconds are required to complete the reaction.

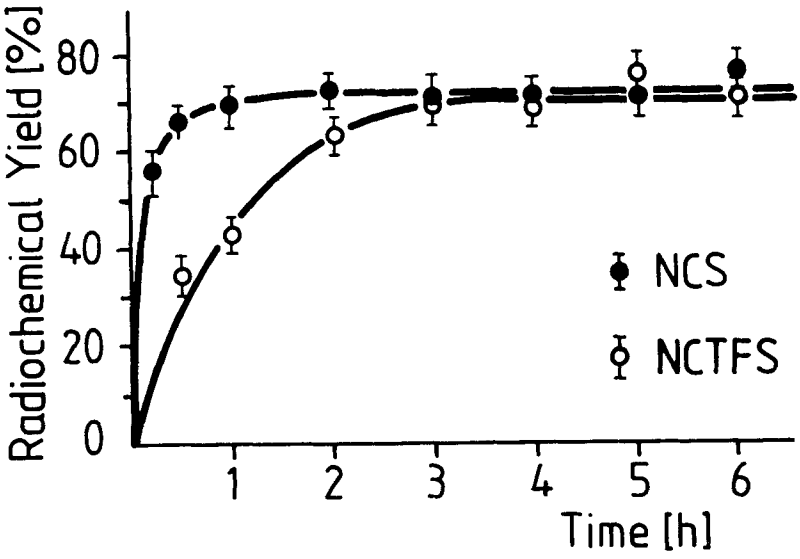

Fig. 1. Dependence of the iodination yield on the reaction time. 2 mg NCS or NCTFS, 50 µl anisole, 5 µl no-carrier-added $Na^{131}I$ solution, 0.5 ml TFAA, room temperature 20-22 °C.

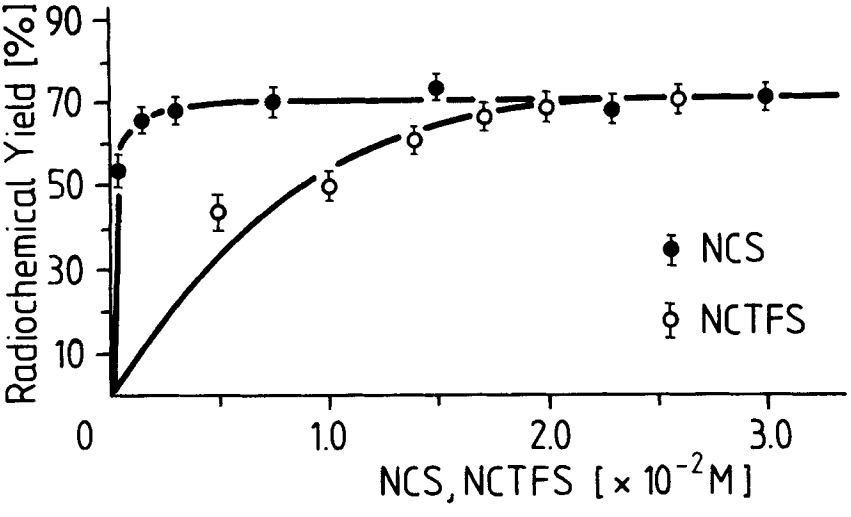

Fig. 2. Dependence of the iodination yield on NCTFS or NCS concentration. 0.5 ml TFAA, 50 µl anisole, no-carrier-added $Na^{131}I$ solution, 4 h reaction time (2 h for NCS), temperature 20-22 °C.

While Chloramine-T gives yields exceeding 70 %, a maximum yield of about 70 % is reached for the radioiodination of anisole by NCS and NCTFS, as shown in Fig. 2. In view of the kinetics (cf. Fig. 1), reaction times of two hours for NCS, and of four hours for NCTFS were chosen to ensure the maximum yields. A reaction time of 30 minutes was used for CAT.

The dependence of the iodination yield on the NCTFS concentration is similar to that obtained in bromination (16). At an NCTFS concentration of about $2 \cdot 10^{-2}$ M, a saturation yield of about 70 % was found. In the case of NCS, a similar saturation yield was observed beginning at only $2 \cdot 10^{-3}$ M NCS. NCS therefore seems to be more efficient than NCTFS, probably due to the high sensitivity of the polyfluorinated N-halosuccinimide towards traces of water.

The dependence of the radiochemical yield on CAT-concentration has already been studied in aqueous solution (18). These results indicate that a concentration of 10^{-3} M CAT is suitable.

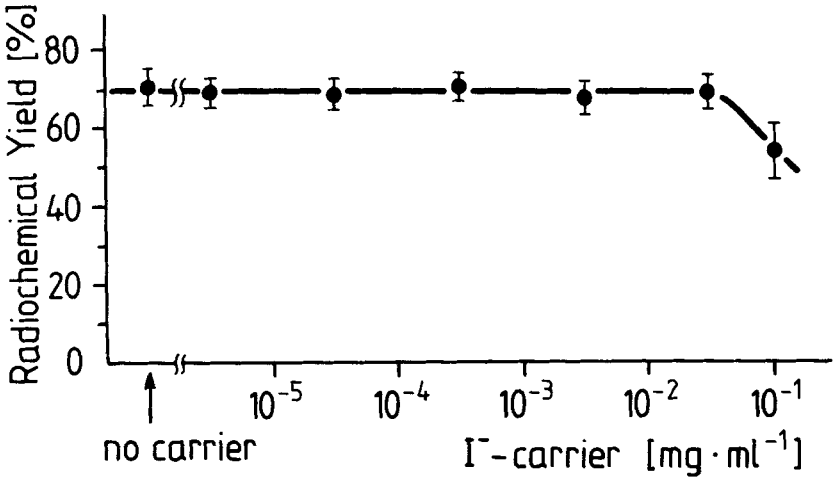

Fig. 3. Dependence of the iodination yield on I^- carrier concentration. 2 mg NCS, 0.5 ml TFAA, 50 µl anisole, 5 µl $Na^{131}I$ solution, temperature 20-22 °C, 2 h reaction time.

The influence of iodide carrier on the radiochemical yield using NCS is illustrated in Fig. 3. The results indicate that a change in iodide concentration from $5 \cdot 10^{-6}$ to $5 \cdot 10^{-2}$ mg/ml does not affect the radiochemical yield. This demonstrates that NCS, and suggests that NCTFS and CAT, are well suited for no-carrier-added radioiodination. An increase of iodide-carrier above about $5 \cdot 10^{-2}$ mg/ml obviously causes a decrease of the radiochemical yield.

In Fig. 4 the dependence of the radiochemical yield on the anisole concentration (a) and on the amount of H_2O from the aqueous radioiodide solution (b) are shown. As expected, Fig. 4a indicates that a further increase of anisole which is already present in considerable excess has little or no effect on the radiochemical yield. Fig. 4b shows that under our experimental conditions about 30 µl of the original aqueous radioiodide solution can be used with no decrease in the radiochemical yield. Thus, experiments can be carried out with a few mCi radioiodine in aqueous solution without evaporating the original radioiodine solution. However, above about 30 µl of water the radiochemical yield decreases drastically, probably due to the hydrolysis of NCTFS.

It should be noted that the chlorinating side-reaction also depends on the amount of water added. When working in pure TFAA chlorination can be completely suppressed (19). The chlorination yield depends on the reactivity of the aromatic substrate and the concentration of the oxidant, and small concentrations of chlorinated by-products were observed only in the case of anisole. Although the chlorinated products can often be separated from the iodinated products, this may represent a problem in the halogenation of large biomolecules, where a separation of by-products

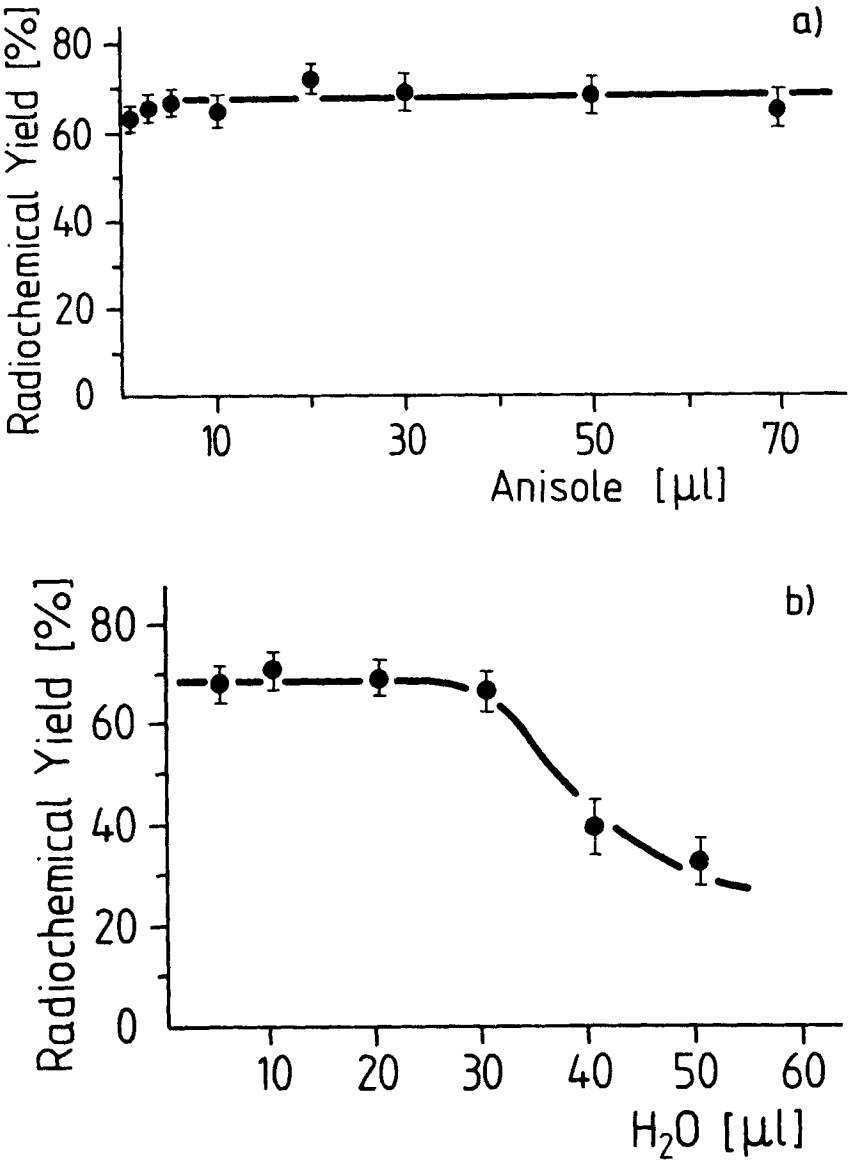

Fig. 4. Dependence of iodination yield on the amount of anisole (a) and H_2O (b).

a) 2 mg NCTFS, 0.5 ml TFAA, 5 µl no-carrier-added $Na^{131}I$ solution, 4 h reaction time, temperature 20-22 °C.

b) 2 mg NCTFS, 0.5 ml TFAA, 50 µl anisole, 4 h reaction time, temperature 20-22 °C.

is difficult or impossible. In this study, we have not specifically tried to minimize chlorinated products. However, minimization of these by-products can easily be accomplished by choosing the appropriate reaction conditions (19).

The radioiodination yields and the isomer distribution of anisole using NCTFS, NCS, NBS and CAT are compared in Table I. The results are very similar as far as the isomer distribution and the radiochemical yields of the N-chloro reagents are compared. As expected, no difference is observed between ^{123}I, ^{125}I and ^{131}I which may contain different amounts of inactive iodine impurities.

Table I

Radiochemical iodination yield and relative o-, m-, p-isomer distribution of anisole using NCTFS, NCS, NBS and CAT (iodination reagent in 0.5 ml TFAA; 50 µl anisole, 5 µl no-carrier-added radioiodide solution; reaction time: see footnote; 20-22 °C).

Reagent	Radiochemical Yield H-substitution (total) (%)	Relative Isomer Distribution (o + m + p = 100)	
NCTFS*	69.3 ± 0.7 (^{131}I)	o-	22.3
		m-	-
		p-	77.7
	63.8 ± 0.7 (^{125}I)	o-	25.3
		m-	-
		p-	74.7
	69.4 ± 1.0 (^{123}I)	o-	26.6
		m-	-
		p-	73.4
NCS**	72.4 ± 4.3	o-	21
		m-	-
		p-	79
NBS**	15.3 ± 3.4	o-	26
		m-	-
		p-	73
Chloramine-T***	75.4 ± 7.9	o-	20.9
		m-	-
		p-	79.1

* 4 h reaction time, 2.0 mg NCTFS

** 2.0 h reaction time, 2.0 mg NCTFS

*** 0.5 h reaction time, 1.0 mg CAT.

The considerably lower yield in the case of NBS is probably due to the competing bromination process, since the highest by-product yield was observed in this system. For this reason NBS does not seem to be suitable for introducing radioiodine into aromatic substrates.

Since radioiodination with CAT in aqueous solution is limited to strongly activated benzene derivatives, we extended our studies to less activated benzenoid systems. Table II shows the radiochemical yields for three different monosubstituted aromatic substrates. Expectedly, the yields decrease when going from anisole to toluene and benzene. As in the case of bromination (16,18,19), the yields are strongly dependent on the activation of the aromatic ring, thus indicating an electrophilic halogenation mechanism. Although we observed no carrier effect in the anisole system, it should be noted that in the case of the less reactive systems toluene and benzene the radiochemical yield increases when carrier is added (19).

Table II

Comparison of the radiochemical iodination yields (saturation) in % of anisole, toluene, and benzene using NCTFS, NCS, and CAT (2.0 mg iodination reagent, 0.5 ml TFAA, 20 µl substrate, 5 µl no-carrier-added $^{131}I^-$-solution, 20-22 °C).

Substrate	NCTFS (4 h)	NCS (4 h)	CAT (10 min)
Anisole	69 ± 5	72 ± 4	75 ± 8
Toluene	47 ± 8	30 ± 6	49 ± 8
Benzene	∿4	∿1	∿3

CONCLUSION

This study shows that N-chlorotetrafluorosuccinimide (NCTFS), N-chlorosuccinimide (NCS) and Chloramine-T (CAT) are equally effective for no-carrier-added iodination in an aprotic solvent such as TFAA. CAT has been previously used only in aqueous solution or in aqueous mixtures. Thus, the procedure described here allows an extension of these iodination methods to compounds which are water-insoluble but are soluble in TFAA. The advantages of CAT in TFAA over water-sensitive NCTFS are also obvious: faster reaction, lower amount of reagent required, and much greater ease of handling. CAT is therefore superior to both NCS and NCTFS as far as the iodination of simple activated monosubstituted benzenes in TFAA are concerned. Differences are to be expected for other substrates, however, especially for more complicated molecules. Thus, the optimization of reaction conditions is mandatory for each individual iodination substrate.

REFERENCES

1. Myers W.G. and Anger H.O. - J. Nucl. Med., 3 pp 183 (1962)
2. Myers W.G., Anger H.O., Lamb J.F. and Winchell H.S. - ^{123}I for application in diagnosis. In Radiopharmaceuticals and Labelled Compounds. Vol. 2 IAEA, Vienna, 249 (1973)
3. Stöcklin G. - Int. J. appl. Rad. Isotopes 28: 131-147 (1977)
4. Qaim S.M., Stöcklin G. and Weinreich R. - "Iodine-123 in Western Europe" Panel discussion, Jül-Conf-20 (1976)
5. Hunter W.M. and Greenwood F.C. - Nature 194: 495 (1962)
6. Silvester D.J. and White N.D. - Nature 200: 65 (1963)
7. Caro R.A., Ciscato V.A., De Giacomini S.M.V., Quiroga S. - Int. J. app. Radiat. Isotopes 26: 527-532 (1975)
8. Greenwood F.C. and Hunter W.M. - Biochem. J.: 89-114 (1963)
9. Hadi U.A.M., Malcolme-Lawes D.J. and Oldham G. - Int. J. appl. Radiat. Isotopes 29: 621-623 (1978)
10. Hadi U.A.M., Malcolme-Lawes D.J. and Oldham G. - Int. J. appl. Radiat. Isotopes 30: 709-712 (1979)
11. Krohn K., Sherman L. and Welch M. - Biochim. Biophys. Acta 285: 404-413 (1972)
12. Lambrecht R.M., Mantescu C., Redvanly C. and Wolf A.P. - J. Nucl. Med. 13: 266 (1972)
13. Hughes W.L., Jr. and Straessle R. - J. Amer. Chem. Soc. 72: 452 (1950)
14. El-Garhy M. and Stöcklin G. - Radiochem. Radioanal. Letters 18(5) 281-290 (1974)
15. Lambrecht R.M. and Wolf A.P. - "Dynamic Studies with Radioisotopes in Medicine" IAEA, Vienna, p. 144 (1975)

16. Coenen H.H., Machulla H.-J. and Stöcklin G. - J. Lab. Comp. Radiopharm. 16: 891 (1979)

17. Coenen H.H., El-Wetery A.S. and Stöcklin G. - J. Lab. Comp. Radiopharm. 18: 114 (1981)

18. Petzold G. and Coenen H.H. - J. Lab. Comp. Radiopharm. 18: 1319 (1981)

19. Coenen H.H. - Thesis University of Cologne, Report Jül-1590 (1979)

20. Wilbur D.S., Bentley G.E. and O'Brien H.A. - J.Lab.Comp. Radiopharm. 18: 1693 (1981)

21. Djerassi J.C. - Chemical Reviews 43: 271 (1948)

22. Lambert F.L., Ellis W.D. and Parry R.J. - J. org. Chem. 30: 304 (1965)

23. Zimmmer W.F., Jr. - Ph.D. Thesis, The Ohio State University, Columbus (1952)

24. Michael H., Rosezin H., Apelt H., Blessing G., Knieper J. and Qaim S.M. - Int. J. appl. Radiat. Isotopes 32: 581 (1981)

25. Stöcklin G. and Tornau W. - Radiochim. Acta 9: 95 (1968)

26. Knust E.J. and Schüller M. - J. Chromatogr. 114: 207 (1975)

27. Vasaros L., Machulla H.-J. and Tornau W. - J. Chromatogr. 62: 458 (1971)