PREPARATION AND HIGH PRESSURE LIQUID CHROMATOGRAPHY OF 5-ASTATOURACIL

G.-J. Meyer, K. Rössler and G. Stöcklin
Institut für Chemie der Kernforschungsanlage Jülich GmbH,
Institut 1 : Nuklearchemie, D-517 Jülich, FRG
Received on December 29, 1975
Revised on February 24, 1976

SUMMARY

5-astatouracil was prepared via the decomposition of the 5-diazonium salt of uracil in the presence of $^{211}At^-$ with overall yields ranging from 20 to 30%. Identification and separation was carried out on four different high pressure liquid chromatography columns. Under identical conditions, astatination is more effective than iodination, and complex formation of halide with the diazonium compound seems to be the product forming mechanism.

KEY WORDS: 5-Astatouracil, Astatine-211, HP Liquid Chromatography

INTRODUCTION

The α-emitter ^{211}At (T = 7.2 h) is of potential importance for radiation biological and therapeutical applications. The specific interest lies in its favourable decay properties (1) and in the fact that the biochemical behaviour of this heaviest halogen is similar to that of iodine. This facilitates the choice of chemical forms in which ^{211}At might be administered for a selective incorporation. Inorganic forms of ^{211}At, mostly At^-, have been used in nuclear medicine, e.g. for studies of thyroidal uptake (2-5), incorporation into tumors and the influence on their growth (6,7) and effects on embryotoxicity (8). Besides a certain enrichment in the thyroid gland and in tumors, the major amount of the radioactivity is distributed in the body rather unspecifically (3,7). Thus, for further studies it seems necessary to prepare organic ^{211}At-compounds, expecially biomolecules, which might act as a carrier to specific organs or centers of disease. Early attempts to label tyrosine and proteins followed the well known methods of iodination, i.e. reaction with N-succinimide, chloramine-T, oxidation processes via H_2O_2 or electrochemical procedures (9,10). A real breakthrough was obtained with the application of ^{211}At-labelled anti-

gens for suppression of immunological defense mechanisms (11-14).

The oxidative labelling methods which have been applied to macromolecules such as lymphocytes or albumins were rather unspecific. There is no information available on the mechanism, on the exact position of the astatine or on the stability of the products formed. Thus, these procedures giving rise to a more or less random astatination, are not appropriate to prepare products for detailed biochemical studies or for potential applications to therapy in man.

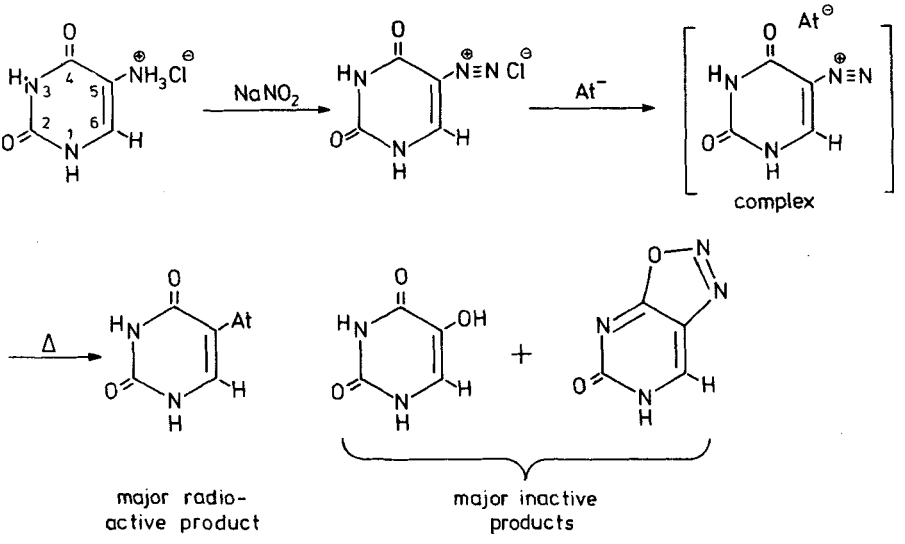
It was the aim of this work to demonstrate a specific astatination in a predictable position. Uracil was chosen as a substrate since it represents a simple pyrimidine base constituent of nucleosides such as thymidine or deoxyuridine. The underlying idea is to use these DNA-precursors as a vehicle for ^{211}At to centers with enhanced cell proliferation such as tumors (15,16). The specific labelling of the pyrimidine ring by carrier-free radioiodine in 5-position had been achieved by various methods. Furthermore, it is possible to check the position of the label by sequential analysis with the corresponding 5-halouracils by high pressure liquid chromatography (16,17).

Previous attempts to astatinate deoxyuridine and uracil by methods analogous to those of iodination were not very successful (15-17). Yields ranging from 0.5 to 2 % just enabled qualitative sequential analysis and elaboration of separation and purification procedures. Obviously, the electrophilic reactivity of At-species is much smaller than that of iodine species. Thus, it was necessary to choose a mechanistically different pathway of halogenation, e.g. the decomposition of diazonium salts of the substrate. This method is similar to the well known Sandmeyer reaction and had already been successfully applied to the astatination of benzoic acid and benzene (10,18) and in a preliminary experiment to serum albumin (10). Recently, the o-, m-, p-astatohalobenzenes (AtX-Ø, X = F, Cl, Br, I) have been prepared by us with good yields (20-30 %) via the decomposition of the diazonium salts of the corresponding o-, m-, p-haloanilines (19). It now seemed promising to use this method for the preparation of 5-astatouracil starting with 5-aminouracil. It was also interesting to obtain information on the relative reactivity of $^{211}At^-$ and $^{125}I^-$ in a competition experiment.

EXPERIMENTAL

^{211}At was produced via the $^{209}Bi(\alpha,2n)^{211}At$-process and converted into the At^--form as described previously (16,17). Reagent grade 5-aminouracil was purchased from Serva-Feinbiochemica, Heidelberg. It was purified by dissolution in 0.5 N HCl and precipitation by addition of 0.5 N NaOH. This precipitate was recrystallized from water solution (80 °C). Carrier-free $^{125}I^-$ was produced via the $^{124}Xe(n,\gamma)^{125}Xe$ process followed by the decay of the ^{125}Xe in the presence of an aqueous Na_2SO_3 solution (17,20).

The reaction via the decomposition of the diazonium salt is shown in the following scheme:



0.1 mmol of 5-aminouracil was dissolved at about 50 °C in 200 µl 7N HCl. After complete dissolution, the mixture was cooled to -5 °C. 50 µl of a fresh 2.5 N solution of $NaNO_2$ were added dropwise under continuous stirring. 2-3 mg of urea were given to the now clear solution. About 0.1 mCi of carrier-free $^{125}I^-$ or up to 1 mCi of $^{211}At^-$ in 50 µl 0.4 N Na_2SO_3-solution were added. After a few minutes of stirring, the reaction mixture was heated for 1-3 min in a water bath to 80 °C until the solution turned yellow and a gas evolution occured. The mixture was then cooled to about 0 °C. The reddish-yellow precipitate was filtered and washed twice with 100 µl of water. The filtrate con-

taining about 90 % of the radioactivity was analysed by high pressure liquid chromatography (HPLC).

The identification of the carrier-free At-compounds by chromatographic methods necessitates a sequential analysis of the homologous halogen compounds, cf. (16). Uracil and the 5-halouracils (X-U, X = H, F, Br, I) from Serva

Table 1 HPLC-columns and conditions of operation*

column number	I	II	III	IV
type	cation exchanger	anion exchanger	anion exchanger	partition chromatography
packing material	Aminex A7	Aminex A25	Aminex A27	Merckosorb-Si 60 (10 µ)
inner diam., mm	4.0	1.8	1.8	4.0
length, mm	100	250	100	500
eluent	0.05 M $KHSO_4$	2 M HCOOH	10^{-2} M $NaNO_3$	1-butanol, saturated with H_2O
temp., °C	25	25	60	25
pressure, bar	180	50	30	50
flow, $ml \cdot min^{-1}$	0.3	0.3	0.3	0.3

*cf. also (16,20)

Feinbiochemica, Heidelberg, were analysed on four different HPLC-columns described in Table 1. Especially the cation exchange column I proves to be a good tool for the separation of side products of halogenation of uracils and deoxyuridines. The columns were made out of V4A stainless steel tubes and were mounted on the HPL-chromatograph UFC-1000 of Hewlett Packard. The eluents used were spectroscopic grade, and the solutions were degassed prior to operation. The net retention volumes of the macroscopic substances (U, X-U) were determined via a UV-detector at 254 nm; the carrier-free ^{125}I- and ^{211}At-reaction products were continuously monitored by a well type NaI(Tl) scintillation counter

(16). Fractions were also collected and measured separately with a NaI(Tl) scintillation detector in an automatic sample changer (Autogamma, Packard Instruments).

RESULTS AND DISCUSSION

The chromatographic sequence of uracil, 5-fluoro-, 5-bromo- and 5-iodo-uracil on four different HPLC-columns is shown in Fig. 1 together with the radioactivity peaks (dashed peaks) of the carrier-free iodine and astatine products.

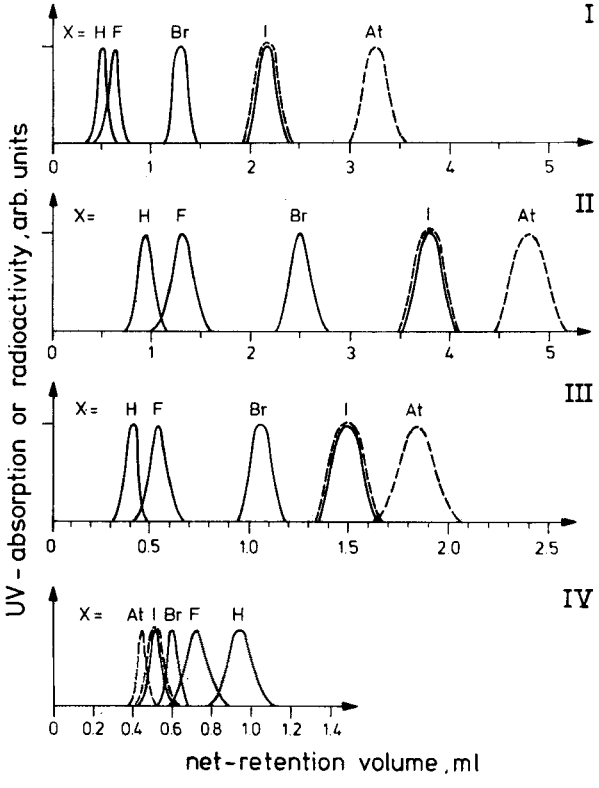


Fig. 1 Sequential analysis of uracil and 5-halouracils (XU; X = H, F, Br, I) and carrier-free radioactive products (dashed peaks) of iodination and astatination on four different HPLC-columns, cf. Table 1.

The peak for ^{125}IU coincides with that of macroscopic amounts of IU on each column. The position of the ^{211}At-product peak fits the sequence of the 5-halouracils quite well on columns I and IV (cation exchange and partition chromatography, respectively). Columns II and III (anion exchange) give retentions which are somewhat smaller than expected. However, within the experimental error, the fitting of the sequence is still reasonably good. Thus, it can be concluded that the major ^{211}At-product formed by decomposition of the 5-diazonium salt of uracil in the presence of At^--ions is indeed 5-astatouracil. Reinjection of the ^{125}IU- and ^{211}At-peak fractions exhibits only one and the same peak again. Storage of the eluate for 0.5 h at 80 °C before reinjection did not change the yield. Obviously, the At-C bond formed is stable under these conditions.

The chromatographic yields of the various columns as obtained from reinjection of the peak fractions are listed in Table 2. They range from 70 to almost 100 %. The cation exchanger (I) gives rise to the best resolution, but also exhibits the lowest chromatographic yields (70 - 80 %). The partition chromatography (IV), on the other hand, gives rise to somewhat better yields, but is also rather unspecific. For preparative purposes it seems advisable to use the anion exchange column (III) which exhibits reasonable resolution and yields. The overall yields on this column for ^{125}IU and ^{211}AtU are listed in Table 3. It should be mentioned that the yields of the individual runs differ very much, since it is difficult to maintain the conditions of the diazonium

Table 2 Chromatographic yields of ^{125}IU and ^{211}AtU on four different HPLC-columns, % of product radioactivity reinjected

	Columns (according to Table 1)			
substance	I	II	III	IV
^{125}IU	>80	>80	>90	~100
^{211}AtU	70-80	80	90	~100

salt decomposition completely constant. The values in Table 3 represent a mean of about 15 runs. It can be seen that the yields of ^{211}AtU are always higher than those of ^{125}IU. This holds for the individual preparations as well as for a competition experiment, in which $^{125}I^-$ and $^{211}At^-$ (both carrier-free) were present during the diazonium salt decomposition. There are, of course, better methods for preparing ^{125}IU; the maximal yield of 30 % for ^{211}AtU, however, is very good, when compared with the yields of other astatination procedures (11,13,17).

Table 3 Overall yields of ^{125}IU and ^{211}AtU on column III, % of radioactivity

substance	separate preparations	competition experiment
^{125}IU	18 ± 3	13 ± 2
^{211}AtU	25 ± 5	20 ± 2

Very low yields ranging from 3 to 6 % are found, when working with weighable amounts of I^- (0.1 mmol). Here, the reaction scheme is complicated by the interaction of macroscopic amounts of I_2 formed during diazonium salt decomposition.

The astatination procedure gives rise to two other unidentified side products, containing 20 to 30 % of the total radioactivity. They are eluted at rather high retention volumes, 10 and 20 ml, respectively, on columns II and III. These peaks cannot be ascribed to simple inorganic At-forms which under the conditions applied remain on the column. The major inactive products are probably 5-hydroxypyrimidine, some of the relatively stable diazouracils (21) and intermolecular coupling products, which precipitate during the diazonium salt decomposition adsorbing about 1 % of the total activity.

In view of the extremely small concentrations of $^{125}I^-$ (about 10^{-10} mol·l^{-1}) and $^{211}At^-$ ($5 \cdot 10^{-12}$ mol·l^{-1}) it seems rather unlikely that the reaction proceeds

via a bimolecular process between an unstable intermediate and a halogen ion. As in the analogous case of the astatohalobenzenes (19) we rather assume that I^- or At^- forms a complex with the diazonium compound (cf. reaction scheme above) which then gives rise to the products upon decomposition. This is also suggested by the increasing tendency of the heavier halogens towards polyvalency, when going to higher rows of the periodic table. Molecular beam experiments on the reactions of Cl with HI and HAt (22,23) also demonstrated that astatine is the stronger polyvalent species and hence has the greater tendency to form complexes. Astatine should therefore exhibit a greater probability for a reaction with the activated 5-position of the pyrimidine ring. This should be independent of the problem whether the reaction is of nucleophilic nature (carbanion formation) - as shown in the above reaction scheme - or of the homolytic (radicalic) type. Only complex formation can explain the fact that astatination proceeds with higher yields than iodination. Otherwise, due to the greater stability of the C-I bond, one would expect the reverse effect.

The high reactivity of At^- observed for this labelling procedure again raises the problem of the low reactivity of oxidized forms of At as compared to those of iodine, cf. (15-17). In contrast to the nucleophilic or homolytic direct attack to the 5-position, the electrophilic attack of oxidized halogen proceeds via addition of HOX to the 5-6 double bond of the pyrimidine ring (24,25). Astatine seems not to form hypoastatous acid (HOAt) but stabilizes probably as a cation At^+ (16). This species, however, seems to be too unreactive for a direct electrophilic attack to the 5-position of uracil and also may form complexes with the solvent, thereby, further decreasing its reactivity.

The decomposition of corresponding diazonium salts lends itself as a tool for the labelling of similar nucleosides with ^{211}At in specific positions. Experiments to label 5-astatodeoxyuridine are under way, but suffer from difficulties in preparation and purification of the starting material 5-aminodeoxyuridine. Organ distribution of inorganic forms of ^{125}I and ^{211}At (7), as well as of 5-iodo- and astatouracils, cf. (26), and - deoxyuridines are presently checked in NMRI-mice, both in normal animals and those with experimental tumors. The results will be reported elsewhere.

Acknowledgement: The authors wish to thank the staff of the cyclotron of the Gesellschaft für Kernforschung GmbH, Karlsruhe for the irradiation of Bi-targets. They are also indebted to Dr. E.J. Knust for a helpful discussion and Mr. G. Eich for experimental assistance.

REFERENCES

1. Jardine, L.J., Phys. Rev. C 11, 1385 (1975).
2. Hamilton, J.G. and Soley, M.H., Proc. Nat. Acad. Sci. 26, 483 (1940).
3. Hamilton, J.G., Asling, C.W., Garrison, W.M. and Scott, K.G., Univ. Calif. Publ. Pharmacology 2, 283 (1953).
4. Hamilton, J.G., Durbin, P.W., Asling, C.W. and Johnston, M.E., in "Peaceful Uses of Atomic Energy", U.N. New York, 1956, 10, p. 175.
5. Basson, J.K. and Shellabarger, C.J., Rad. Res. 5, 202 (1956).
6. Ueda, G. and Mori, T., Amer. J. Path. 51, 601 (1967).
7. Persigehl, M. and Rössler, K., AED-CONF-75-193-078 (1975).
8. Borrás, C., Brent, R.L., Gorson, R.O. and Lamb, J.F., Proc. 1st World Congress of Nuclear Medicine, Tokyo, 1974, p. 335.
9. Hughes, W.L. and Gitlin, D., BNL Quart. Progr. Report - 314, p. 48 (1954) and Fed. Proc. 14, 229 (1955).
10. Hughes, W.L. and Klinenberg, J., BNL Quart. Progr. Report - 367, p. 42 (1955).
11. Neirinckx, R.D., Myburgh, J.A. and Smit, J.A., Radiopharmaceuticals and Labelled Compounds, IAEA Vienna 1973, Vol. 2, 180.
12. Smit, J.A., Myburgh, J.A. and Neirinckx, R.D., Clin. exp. Immunol. 14, 107 (1973).
13. Aaij, C., Tschroots, W.R.J.M., Lindner, L. and Feltkamp, T.E.W., Int. J. Appl. Rad. Isotopes 26, 25 (1975).
14. Samson, G., in "Nuklearmedizin", ed. H.W. Papst, F.K. Schattauer Verlag, Stuttgart 1974, p. 506.
15. Rössler, K., Tornau, W. and Stöcklin, G., AED-CONF-72-350-002 (1972).
16. Rössler, K., Tornau, W. and Stöcklin, G., J. Radioanalyt. Chem. 21, 199 (1974).

17. Meyer, G.-J., Report-Jül-1076-NC (1974).

18. Samson, G. and Aten, A.H.W., Radiochim. Acta 13, 220 (1970).

19. Meyer, G.-J., Rössler, K. and Stöcklin, G., Radiochem. Radioanalyt. Letters 21, 247 (1975).

20. Machulla, H.-J., Laufer, P. and Stöcklin, G., J. Radioanalyt. Chem., in press

21. Whittaker, N., J. Chem. Soc. 1951, 1565.

22. Grover, J.R. and Iden, C.R., J. Chem. Phys. 61, 2157 (1974).

23. Grover, J.R., Iden, C.R., Schubert, F.E., Muckermann, J.T. and Watanabe, T., Proc. IX. Internat. Conf. Physics of Electronic and Atomic Collisions, Seattle, July 24-30, 1975, No. 331.

24. Wang, S.Y., J. org. Chem. 24, 11 (1959).

25. Brown, D.J.,"The Pyrimidines", Interscience Publ. Inc., New York 1962.

26. Meyer, G.-J., Rössler, K. and Stöcklin, G., AED-CONF-75-404-029 (1975).