THE PREPARATION OF AROMATIC ASTATINE COMPOUNDS THROUGH AROMATIC MERCURY COMPOUNDS PART II: ASTATINATION OF PYRIMIDINES AND STEROIDS

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SUMMARY

Several ^{211}At-astatopyrimidines (5-At-uracil, 5-At-cytosine and their nucleosides and nucleotides including DNA and RNA) have been synthesized in high radiochemical yields by reaction of $^{211}At/I_2$ and the corresponding chloromercury compounds. Also some astato-steroids (6-At-cholesterol and 2- and 4-astatoestradiol) have been prepared by this method. The stability in vitro was determined under different conditions in comparison with the analogous iodo compounds.

Key words: Astatine 211, electrophilic astatination, mercury compounds, At-pyrimidines, At-steroids

INTRODUCTION

In an earlier publication [1] we reported on the preparation of aromatic astatine compounds from chloromercury derivatives and $^{211}At/I_2$. Under mild conditions and in high radiochemical yields (70-95%) the astato derivatives were obtained. We have further tested the applicability of this method and this paper deals with the synthesis of biomedical interesting compounds such as At-steroids, 5-At-uracil, 5-At-cytosine and some of their nucleosides and nucleotides as well as stability measurements of these compounds in comparison with the analogous iodo derivatives.

EXPERIMENTAL

^{211}At was prepared by the $^{209}Bi(\alpha,2n)^{211}At$ reaction at the synchrocyclotron of the Free University of Amsterdam or at the cyclotron of the KVI in Groningen.
It was isolated as ^{211}At-astatide in diluted NaOH as described earlier [2].
^{131}I-iodide was obtained from The Radiochemical Centre, Amersham, in

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aqueous NaOH without reducing agents.The activities were measured in a NaI(Tl) well-type crystal on the 365 keV gamma-rays of ^{131}I or on the Po X-rays of ^{211}At.
DNA (from calf thymus)and RNA (from Yeast) were purchased from Serva.

Mercuration:

Uracil was mercurated with $HgSO_4$ as described elsewhere [1]. The other pyrimidines, including DNA and RNA, were mercurated by reaction with $Hg(OAc)_2$ in NaOAc-buffer (pH = 5) at 50 °C for 3-5 hours according to Dale [3]. The compounds were converted into the chloromercury derivatives by reaction with concentrated NaCl. In the case of soluble chloromercury compounds, these derivatives were precipitated by addition of ethanol. The chloromercury pyrimidines were isolated as white amorphous powders.
Estradiol was mercurated by reaction with $Hg(OAc)_2$ in ethanol/water during 16 hours at room temperature. After addition of NaCl the precipitate was washed with water and dried on P_2O_5. Cholesterol was mercurated and purified according to Merz [4].

Astatination (iodination):

The astatination of uracil has been described elsewhere [1]. The pyrimidines were astatinated in NaOAc-buffer (0.5 M, pH = 5). HgCl-pyrimidine (10 µmole) was suspended in 0.5 ml of buffer and the ^{211}At-activity (or ^{131}I-activity) was added, followed by 0.9 eq. of KI_3 in steps of 0.1 eq. in a period of 5 minutes. The mixture was stirred for 1 hour and the precipitated HgI_2 was dissolved by adding an excess of KI.
The astatination (iodination) of steroids was performed in $CHCl_3$ for 1 hour. The mercurated steroid (10 µmole) in 0.5 ml of $CHCl_3$ was stirred vigorously with the ^{211}At (or ^{131}I) activity in diluted aqueous H_2SO_4 and after 5 minutes 0.9 eq. of KI_3 was added and stirring was continued for 1 hour. The $CHCl_3$ layer was separated and extracted with aqueous KI- and Na_2SO_3-solutions to remove Hg-salts and inorganic astatine and iodine respectively.

Isolation and purification:

The astatinated pyrimidines, except UTP, DNA and RNA, were isolated by chromatography of the reaction mixture over DEAE-sephadex using 0.9% NaCl as eluent. 5-At-UTP was purified by electrophoresis of the reaction mixture (Whatmann-3 MM-paper, citrate buffer, 30 mM, pH = 4, 15 min, 75 V/cm) and isolated by extraction of the paper with 0.9% aqueous NaCl.

At-RNA and DNA were isolated and purified by gelfiltration over sephadex (PD-10-columns) with 0.9% NaCl as eluent.
The astatinated steroids were purified by chromatography over SiO_2 with $CHCl_3$ as eluent.
The astatinated compounds were identified by thin-layer chromatography and/or electrophoresis (Table I and II). The position of the mass peaks was determined by measuring the UV absorption or in the case of the steroids by reaction with iodine-vapour.

Stability measurements:

The stability of the 5-At-pyrimidines was determined with paper-electrophoresis and TLC. No decomposition was observed under the conditions of the analysis. The stability of At-RNA and At-DNA was measured by means of gel-filtration or by precipitation of the nucleic acids with ethanol.
The stability of the steriods was determined by TLC on SiO_2.

RESULTS AND DISCUSSION

Astatination of the pyrimidines

Meyer et al. [5] prepared the biological interesting [6] 5-astatodeoxyuridine (Fig. 1, R_1 = OH, R_2 = R_3 = H) by reaction of ^{211}At-astatide with the 5-diazonium salt of deoxyuridine. However, the yield was very low (3%) due to side-reactions such as the cleavage of the sugar-pyrimidine bond by the strong acidic reaction conditions. We have reported already on the synthesis of 5-At-uracil by the chloromercury method [1]; this compound was obtained in a very clean reaction in 85% yield, which exceeded the yield of the diazonium-method (25%) [7].
We decided to test also the astatination through the mercury compounds of the nucleosides and nucleotides of uracil (Fig. 1, R_1 = OH) and cytosine (R_1 = NH_2). Especially 5-At-cytosine and derivatives cannot be synthesized from diazonium compounds.

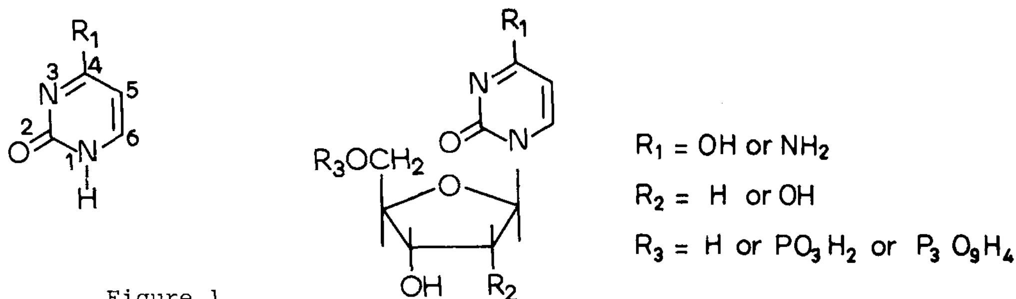

Figure 1

The mercuration of nucleosides and nucleotides, including DNA and RNA, has been studied extensively by Dale et al. [3]. Acetoxy mercuration at the 5-position of the pyrimidines is possible by reaction with $Hg(OAc)_2$ in acetate-buffer at pH = 5-8. By exchange of the acetoxy group for chlorine the reactive 5-chloromercury derivatives are obtained. These derivatives can be converted into the corresponding ^{211}At-5-astato derivatives in yields of 70 - 95% by reaction with $^{211}At/KI_3$; the analogous ^{131}I compounds can be prepared in lower yields (about 25%) by reaction with $^{131}I/KI_3$. A summary of the synthesized astato-products and the radiochemical yields is given in Table I. Also the chromatographic behaviour of the astato-pyrimidines in comparison with the ^{131}I derivatives is given. In general the R_F-values of the At-pyrimidines are somewhat lower than those of the corresponding iodo derivatives. This can be explained from the weaker field effect of astatine with respect to iodine resulting in a higher pK_a-value for the At-pyrimidine than that of the corresponding iodo derivatives [8].

Astatination of steroids

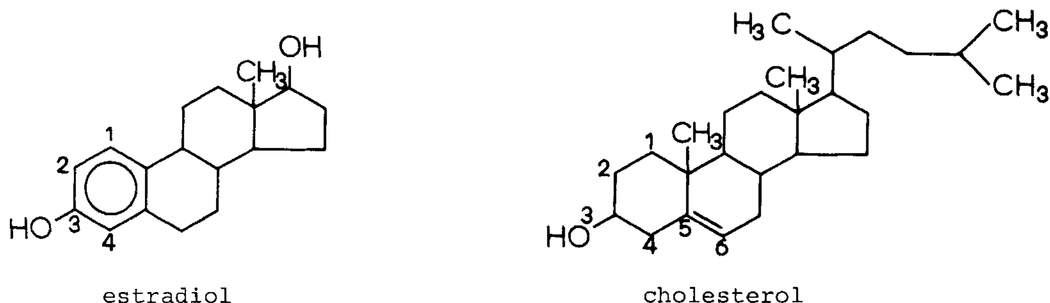

Figure 2

We also attempted the astatination of the steroids estradiol and cholesterol (Fig. 2). Estradiol is a substituted phenol and therefore it can easily be mercurated with $Hg(OAc)_2$ at room temperature. The position of the mercury atom in the steroid was determined by reaction with $^{131}I/KI_3$ and analysis of the iodinated products.
In a total radiochemical yield of 25% 2-iodo-, 4-iodo- and 2,4-diiodoestradiol were isolated in a ratio of 3 : 1 : 1; for comparison electrochemical iodination [9] results in a ratio of about 4 : 4 : 1. Astatination with $^{211}At/KI_3$ gave also three products in a radiochemical yield of 95%. These were identified as 2-astato-, 4-astato- and 2,4-astatoiodoestradiol (ratio 3 : 1 : 1) on basis of their R_F-values (Table II).
The mercuration of cholesterol, which is not an aromatic compound,

has been studied by Levin et al. [10]. The Hg atom is introduced in the 6-position of the steroid and the resulting compound can be converted into the very stable 6-iodocholesterol by reaction with I_2 [10]. Astatination of 6-chloromercurycholesterol resulted in ^{211}At-6-astatocholesterol in a yield of 95%.

Table I Yields and properties of some At-pyrimidines

Product	Radioch.yield	Characterisation	
		TLC-R_F-values	electrophoresis-mobilities
5-At-uracil	80-90%	A:0.57 (^{131}I:0.59)	
5-At-uridine	75-85%	A:0.34 (^{131}I:0.39) B:0.58 (^{131}I:0.63)	C: 3-5 cm (30 min)
5-At-deoxyuridine	80-90%	A:0.48 (^{131}I:0.55) B:0.56 (^{131}I:0.60)	C: 3-5 cm (30 min)
5-At-UMP	75-85%	B:0.50 (^{131}I:0.51)	E: 13-15 cm (30 min)
5-At-UTP	65-75%	B:0.65 (^{131}I:0.66)	D: 10-12 cm (15 min)
5-At-cytosine	80-90%	not determined	F: 12-15 cm (8 min)
5-At-cytidine	70-80%	A:0.12 (^{131}I:0.14) B:0.48 (^{131}I:0.52)	F: 15-17 cm (20 min)
5-At-CMP	75-85%	B:0.50 (^{131}I:0.52)	F: 10-12 cm (30 min)
5-At-dCMP	70-80%	B:0.70 (^{131}I:0.72)	F: 6-8 cm (30 min)
At-RNA	92-98%	-	-
At-DNA	91-94%	-	-

A: SiO_2: organic phase of benzene/H_2O/1-butanol (5 : 4 : 3)
B: cellulose: ethanol/water (7 : 3)
C: 75 V/cm, citrate-buffer, 30 mM, pH = 4, to cathode
D: 75 V/cm, citrate-buffer, 30 mM, pH = 4, to anode
E: 110 V/cm, H_2O, CH_3COOH, HCOOH:8.5; 1.5; 0.5; to anode
F: 110 V/cm, H_2O, CH_3COOH, HCOOH: 8.5; 1.5; 0.5; to cathode
UMP: uridinemonophosphate; UTP: uridinetriphosphate
CMP: cytidinemonophosphate; dCMP: deoxycytidinemonophosphate

Table II Yields and properties of some astato-steroids

Product	Radioch. yield	Characterisation TLC R_F-values
2-astatoestradiol	55%	A: 0.35 (^{131}I: 0.35)
4-astatoestradiol	19%	A: 0.29 (^{131}I: 0.29)
2,4-astatoiodoestradiol	18%	A: 0.43 (^{131}I: 0.43)
6-astatocholesterol	92-95%	B: 0.32 (^{131}I: 0.33)

A: SiO_2: $CHCl_3$/ethylacetate/methanol (100 : 10 : 1)
B: SiO_2: $CHCl_3$

Stability of the pyrimidines

We measured the stability of the different astatopyrimidines in comparison with the analogous iodopyrimidines. In all cases investigated the astatopyrimidines behaved similar to the iodc derivatives and no indications for a weak C-At bond were found.
5-At-uracil, 5-At-cytosine and their nucleosides were stable at room temperature at pH = 1, 4, 7 or 11.5 and at 50 °C at pH = 1, 4 or 7 as measured over a period of 20 hours. Heating at 50 °C at pH = 11.5 resulted in loss of the halogen-substituent (as well for ^{211}At as for ^{131}I; 20-30% after 18-22 h) probably by a direct attack of OH^- on the 5-position of the pyrimidine nucleus [11,12a].
Also dephosphorylation was observed with the nucleotides. At-uridine-monophosphate decomposed (for 50%) into At-uridine by heating at 50 °C for 20 hours at pH = 1. 5-Astato-uridinetriphosphate was, like the parent compound, UTP, even more sensitive to hydrolysis of the phosphate-group. During the mercuration and subsequent astatination about 10% 5-At-UMP is formed together with ≈ 70% 5-At-UTP. Heating of 5-At-UTP at pH = 7 to 70 °C resulted in a fast dephosphorylation leading to 5-At-UMP while under acidic conditions this hydrolysis already occurred at room temperature. At pH = 1 5-At-CMP decomposed for 60% into 5-At-cytidine in 20 hours at 50 °C; a small amount of 5-At-cytosine was found indicating some loss of the sugar-moiety. The sugar-pyrimidine bond in dCMP is less stable under acidic conditions [12a,b]; heating of 5-At-dCMP at 50 °C for 20 hours destroyed this compound completely (formation of 5-At-deoxycytidine and 5-At-cytosine).
We have also tested the stability of the 5-astato-nucleosides and

nucleotides in the presence of sulphite; cytosine and derivatives are converted into uracils by reaction with sulphite (13) while dehalogenation of 5-halogenouracils is possible via the bisulphite adducts (14). Again no difference in reactivity between the astato and iodo derivatives was observed. Incubation with 50 mM $NaHSO_3$ at pH = 7 and room temperature gave a $T_{\frac{1}{2}}$ of $\approx$ 20 hours for the 5-halogeno-cytosine derivatives, while this was decreased to a $T_{\frac{1}{2}}$ of 30 minutes in 1 M $NaHSO_3$ (15). Both 5-At and 5-I-uracils were stable in the presence of 50 mM of $NaHSO_3$ (measured over a period of 20 hours) (15).

Also the stability of the different astatopyrimidines in the presence of H_2O_2 was determined (16).

Again both iodo- and astatocytosine derivatives slowly decomposed with about the same rate (e.g. 30% decomposition for cytosine and CMP after 20 hours in 2 mM of H_2O_2), while no decomposition could be measured for the 5-halogenouracil derivatives.

We performed similar experiments with astatinated RNA and DNA. No de-astatination was observed after incubation at room temperature at different pH's (2 - 11.5) or at pH = 7 with H_2O_2 (2 mM) and sulphite (50 mM) (15,17).

Similar to the nucleosides heating at 50 °C showed only at pH = 11.5 some deastatination (15 - 20% after 20 hours) of the astatinated nucleic acids. Some degradation of the nucleic acids was observed at heating at all pH-values as was determined by gelfiltration. However, the carbon astatine was not broken as was shown by precipitation with ethanol.

Stability of the steroids

o-Halogenophenols are rather reactive compounds which are sensitive to oxidation and dehalogenate quite easily (18). This holds also for iodoestradiol (19). Shida et al. (19a) measured a deiodination of 10% after 72 hours at 4 °C at pH = 7, and we got the same result at 50 °C after 20 hours (15).

We also determined the deastatination of the different astato-estradiols in mixtures of ethanol/aqueous buffers (5 : 1). The results indicated that similarly to the halogenotyrosines (18) the astato-estradiols were less stable than the corresponding iodoestradiols. The measured chemical half-lives of the astatoestradiols were higher than those of the astatotyrosines, because of the presence of ethanol (18). At room temperature no decomposition was observed in acidic medium (0.4 N H_2SO_4) or at pH = 7 for 20 hours. However, on heating to 50 °C at pH = 7 a complete destruction of astatoiodo-

estradiol, and a 75 - 80% deastatination of 2- and 4-astatoestradiol took place. Also addition of H_2O_2 (final concentration 2 mM) resulted in a deastatination of 60% for the astatoestradiols at room temperature after 20 hours. The astatoestradiols as well as the astato-tyrosines [18] are unstable in alkaline solutions; at pH = 11 at room temperature 40% deastatination was observed for astatoiodoestradiol after 20 hours, while 2- and 4-astatoestradiol were again more stable (20% decomposition).
Compared to 19-iodocholesterol or norcholesterol [20], 6-iodocholesterol is extremely stable [10]. This stability is also reflected in 6-astatocholesterol. No decomposition, measured over a period of 20 hours, was observed on heating solutions of At-cholesterol (in ethanol/water = 5 : 1) at 70 °C or by incubation at room temperature with H_2O_2 (20 mM) or sulphite (50 mM).

CONCLUSION

The astatination through chloromercury derivatives is a mild and satisfactory method for the introduction of astatine in organic molecules. As is shown not only aromatic compounds can be astatinated but in principle any substrate that can be mercurated. Furthermore it is clear that in general organic astatine compounds are, at least in vitro, stable molecules.

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