Journal of Chromatography, 81 (1973) 57–63

CHROM. 6699

THE CHEMISTRY OF THE AMINOCHROMES

XIX. THE REACTIONS OF ADRENOCHROME, ADRENOLUTIN AND 5,6-DIHYDROXY-1-METHYLINDOLE WITH SOME SILYLATING AGENTS*,**

R. A. HEACOCK and J. E. FORREST

Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia (Canada)

(Received March 14th, 1973)

SUMMARY

Adrenolutin (*i.e.* 5,6-dihydroxy-1-methylindoxyl) and 5,6-dihydroxy-1-methylindole reacted with the following silylating agents: hexamethyldisilazane (HMDS)–pyridine, N,O-bis(trimethylsilyl)acetamide (BSA) and trimethylsilyldiethylamine (TMSDEA) to give 3,5,6-tri(trimethylsilyloxy)-1-methylindole and 5,6-di(trimethylsilyloxy)-1-methylindole, respectively. Adrenochrome reacted with these three silylating agents to give the trimethylsilyl derivatives of adrenolutin and 5,6-dihydroxy-1-methylindole mentioned above together with other unidentified by-products.

INTRODUCTION

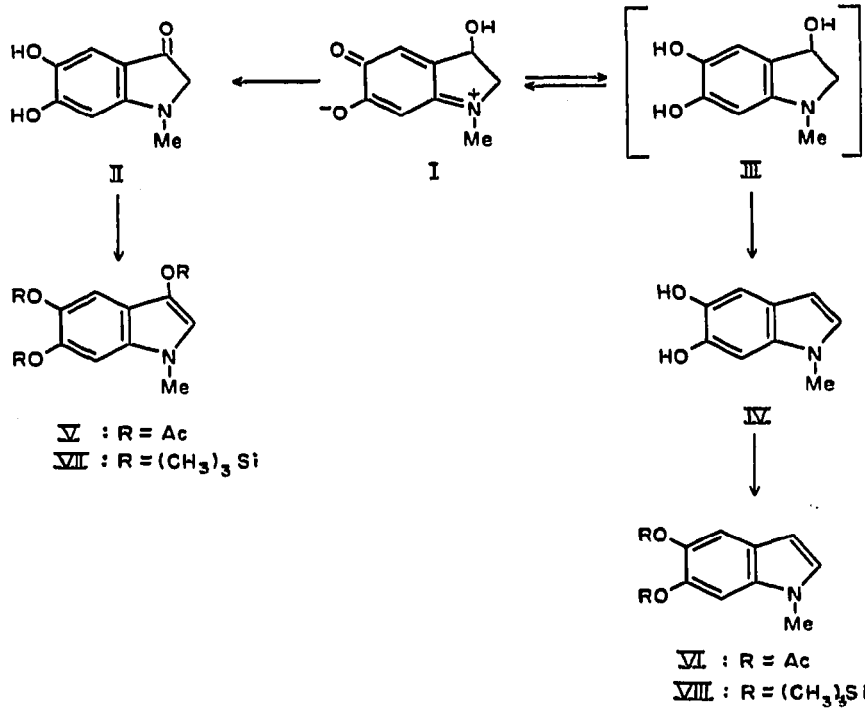
Aminochromes, such as adrenochrome (I) are highly polar compounds with very low volatility; most aminochromes simply decompose without melting on heating. Consequently, it is necessary to convert the aminochromes to stable volatile derivatives before any gas–liquid chromatographic (GLC) analysis of these compounds can be carried out. The formation of trimethylsilyl derivatives has been widely used for converting heat-sensitive polar substances, such as the catecholamines, to products suitable for gas chromatographic (GC) work (*cf.* ref. 1). This technique has also been used to enable proton magnetic resonance (PMR) studies to be carried out on compounds such as catecholamines in non-polar solvents[2].

A brief report[3] appeared in the literature two years ago in which the formation of volatile trimethylsilyl derivatives of adrenochrome (I) and adrenolutin (*i.e.* 5,6-dihydroxy-1-methylindoxyl, II) was used in a GC procedure for the determination of these compounds in purified extracts of plasma obtained from rats, after the animals had been subjected to high oxygen pressure. However, no experimental details for the formation of the trimethylsilyl derivatives were given but it appeared that adrenochrome could be distinguished from adrenolutin by the method used[3].

* Part XVIII, *Can. J. Chem.*, submitted for publication.
** Issued as NRCC No. 13253.

The GLC separations were carried out using glass columns with SE-30 as the stationary phase[3].

Adrenochrome (I) rearranges very readily to form the highly fluorescent product known as adrenolutin (II); this reaction is known to be markedly base-catalysed (*cf.* refs. 4 and 5). Adrenochrome (I) which possesses an *o*-quinone function is also very readily reduced; however, the hypothetical "leucoadrenochrome" (*i.e.* 3,5,6-trihydroxy-1-methylindoline, III) has never been isolated, the major reduction product usually formed in these reactions being 5,6-dihydroxy-1-methylindole (IV)[4,5]. The rearrangement and reduction products II and IV, respectively, are both very reactive and relatively unstable compounds, particularly in solution, being rapidly oxidised on standing to dark insoluble melanin-like products. However, the compounds II and IV both form stable crystalline acetyl derivatives, namely 3,5,6-triacetoxy-1-methylindole (V) and 5,6-diacetoxy-1-methylindole (VI), respectively. The triacetoxy derivative V can be readily obtained directly from either I or II by the action of acetic anhydride and pyridine (*cf.* ref. 5).



By analogy with the reaction of acetic anhydride and pyridine with I (ref. 6), it would be expected that, on treatment with a silylating agent which has some basic character, I would give the tri(trimethylsilyl) derivative VII, the same as the product expected to be formed from II, whilst IV would be expected to give the di(trimethylsilyl) derivative VIII.

The action of three silylating agents: hexamethyldisilazane (HMDS); N,O-bis(trimethylsilyl)acetamide (BSA) and trimethylsilyldiethylamine (TMSDEA) on

adrenochrome (I), adrenolutin (II) and 5,6-dihydroxy-1-methylindole (IV) has now been studied. In the first two cases, the effect of the addition of pyridine to the reaction mixture was also studied. This communication reports the results of these investigations, which may be of some value in future studies on the formation of trimethylsilyl derivatives for GLC analysis of the aminochromes and their derivatives.

EXPERIMENTAL

Chemical

Adrenochrome[7], adrenolutin[8] and 5,6-dihydroxy-1-methylindole[9] were prepared by the methods described in the literature. The silylating agents HMDS, BSA and TMSDEA and the "silylation grade" pyridine were obtained from the Pierce Chemical Co., Rockford, Ill., U.S.A.

The trimethylsilyl derivatives of II and IV were prepared for PMR spectroscopy in the following manner. The indole compound (100 mg) was dissolved in a mixture of HMDS (2 ml) and dry pyridine (2 ml) in an atmosphere of nitrogen. The reaction flask was loosely stoppered and heated, with stirring, on an oil-bath at 80–95° for *ca.* 1 h. The reaction mixture was allowed to cool to room temperature and carbon tetrachloride (B.D.H. "Analar" grade) added, and the solution concentrated *in vacuo*; fresh carbon tetrachloride was added from time to time until all the silylation reagents had been removed. The solution of the silyl derivatives, so obtained, in carbon tetrachloride (1 ml), was suitable for direct determination of their PMR spectra.

Silylation reactions

With HMDS. A sample of freshly prepared adrenochrome, adrenolutin or 5,6-dihydroxy-1-methylindole (5 mg) was treated with a mixture of HMDS (0.1 ml) and pyridine (0.1 ml) in a Pierce "reacti-vial" (0.3 ml total capacity). The vial was stoppered and the reaction mixture vigorously shaken for a few minutes and allowed to stand overnight at room temperature. The products so obtained were suitable for direct GLC analysis. The above procedure was repeated in the case of adrenochrome using HMDS alone.

With BSA. A sample of adrenochrome, adrenolutin or 5,6-dihydroxy-1-methylindole (5 mg) was treated with BSA (0.2 ml) in a vial. The vial was stoppered, vigorously shaken for a few minutes and the products used directly for the GLC analysis. The procedure was repeated in the case of adrenochrome, with the addition of pyridine (0.1 ml).

With TMSDEA. A sample of adrenochrome, adrenolutin or 5,6-dihydroxy-1-methylindole (5 mg) was heated under reflux with TMSDEA (0.2 ml) for 1–1½ h. The reaction mixtures were allowed to cool and used directly for GLC analysis.

Gas chromatography

A Hewlett-Packard Model 5750 dual-column gas chromatograph, operated as a single column instrument fitted with a flame ionisation detector (FID) was used. The operating conditions used for the GC experiments were:

Stationary liquid phase, SE-30 Ultraphase (3%); solid support, 80–100 mesh Chromosorb W, AW, DMCS-treated; column, 6 ft. × ⅛ in., stainless steel; initial

column temperature, 100°; final temperature, 300°; programme rate, 10°/min; detector, FID; injection port temperature, 320°; detector oven temperature, 320°; carrier gas (He) flow-rate, 60 ml/min; hydrogen flow-rate, 45 ml/min; air flow-rate, 100 ml/min; sample size, 2–10 μl.

Combined gas chromatography–mass spectrometry

The Hewlett-Packard Model 5750 instrument described above was coupled to a DuPont Model 21-491 mass spectrometer, via a single stage jet separator. Mass spectra (at *ca.* 70 eV) were recorded to correspond to the peak maxima as shown by the GLC recorder. Spectra were also recorded, as desired, at other positions on the peaks.

The operating conditions used for the gas chromatograph were similar to those described above, with the exceptions of the hydrogen and helium flow-rates which were both 30 ml/min. Samples (10–50 μl) of the reaction mixtures were used for the GLC–mass spectrometric investigation.

RESULTS AND DISCUSSION

Adrenolutin and 5,6-dihydroxy-1-methylindole reacted, not unexpectedly, with the three silylating agents investigated, namely HMDS-pyridine, BSA and TMSDEA, to give 3,5,6-tri(trimethylsilyloxy)-1-methylindole (VII) and 5,6-di(trimethylsilyloxy)-1-methylindole (VIII), respectively, in good yield in both instances. These products were identified on the basis of their mass spectra (see Fig. 1), which showed molecular ions at $M^{+\cdot}$ 395 and $M^{+\cdot}$ 307, respectively, and their PMR

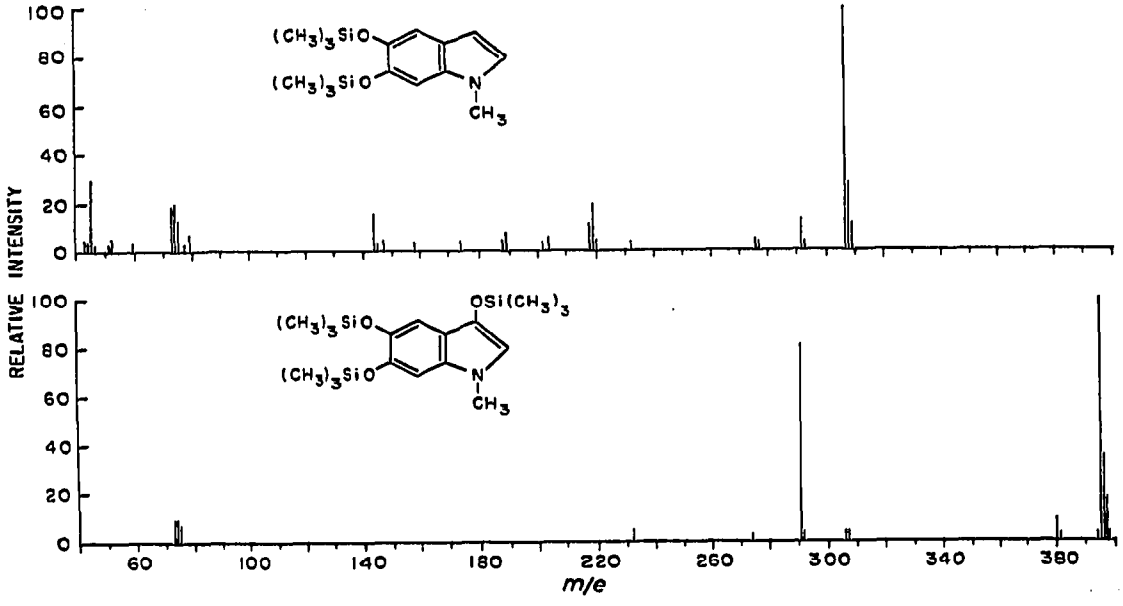


Fig. 1. Mass spectra (at ~ 70eV) of VII (below) and VIII (above). Spectra taken from GLC–mass spectrometry experiments.

spectra which showed the following peaks: VII, δ (CCl_4): 0.21 (27H, s, trimethylsilyl H's); 3.50 (1H, s, N–CH_3); 6.30 (1H, s, 7-H); 6.40 (1H, s, 2-H); 6.76 (1H, s, 4-H) and VIII, δ (CCl_4): 0.21 (18H, two s, trimethylsilyl H's); 3.62 (3H, s, N–CH_3); 6.13 (1H, dd, 3-H, $J_{2,3}$ = 3.2 Hz, $J_{3,7}$ = 1.0 Hz); 6.58 (1H, d, 7-H, $J_{3,7}$ = 1.0 Hz); 6.72 (1H, d, 2-H, $J_{2,3}$ = 3.2 Hz); 6.86 (1H, s, 4-H). Both these products showed only single peaks in the GC analysis (see Fig. 2) indicating the products obtained from II and IV were essentially pure. The different retention times of VII and VIII can be seen from Fig. 2.

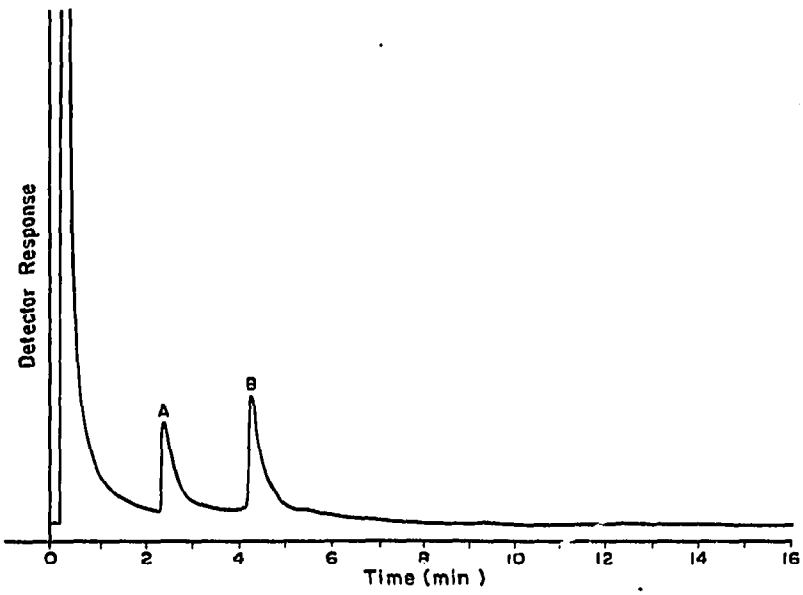


Fig. 2. GLC trace showing the different retention times of the trimethylsilyl derivatives of adrenolutin (II) and 5,6-dihydroxy-1-methylindole (IV) (*i.e.* VII and VIII; peaks B and A on the figure, respectively). The initial column temperature was 100° and the programme rate was 10°/min.

The reactions of adrenochrome with the three silylating agents were of interest especially as the results were more complicated than had been anticipated, particularly in the case of the HMDS–pyridine reaction. By analogy with the smooth conversion of I to V that is brought about by the action of acetic anhydride and pyridine[6] an efficient conversion of I to VII would have been expected in this case. However, GC examination of the dark-coloured products of the adrenochrome–HMDS–pyridine reaction showed two major peaks indicating the presence of both VII and VIII in the reaction mixture. When I was treated with HMDS in the absence of pyridine, a very slow reaction occurred, partly due to its low solubility in the reagent. Two products appeared to be formed, however their retention times suggested that they were neither VII nor VIII. These unknown compounds were not formed in sufficient quantity to enable them to be identified.

The silylating agent BSA is known to silylate certain compounds rapidly in the absence of solvent (*cf.* ref. 1). It was not possible to identify the products of the reaction between I and BSA alone, however in the presence of a small quantity of

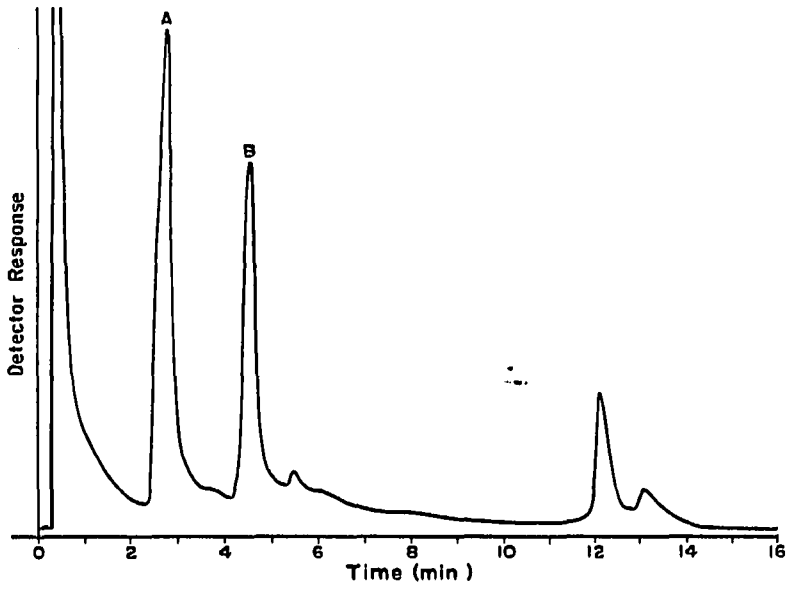


Fig. 3. GLC trace of the products of the adrenochrome–BSA–pyridine reaction mixture. Peak A is due to VIII and peak B is due to VII. The initial column temperature was 100° and the programme rate was 10°/min.

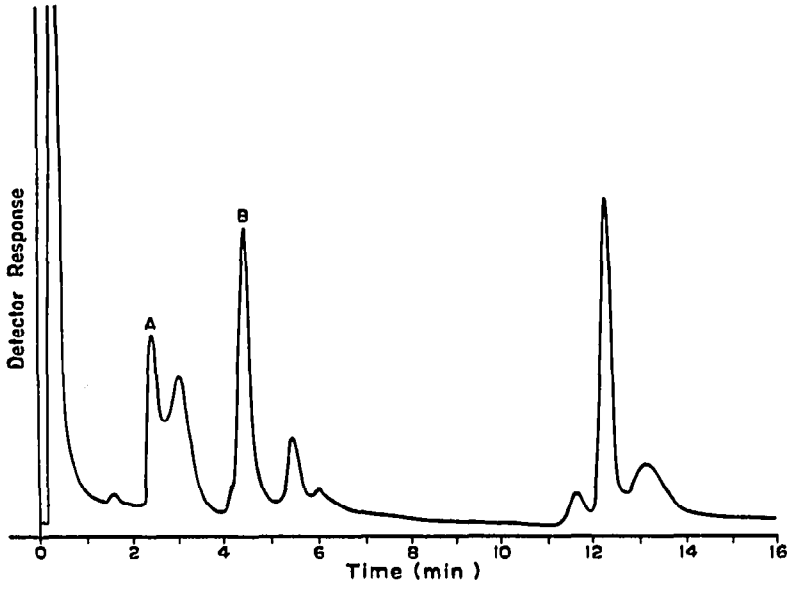


Fig. 4. GLC trace of the products of the adrenochrome–TMSDEA reaction mixture. Peak A is due to VIII and peak B is due to VII. The initial column temperature was 100° and the programme rate was 10°/min.

pyridine, GLC analysis of the reaction mixture showed three main peaks, two being due to VII and VIII; a third product, with a higher retention time was also produced which has not yet been identified (see Fig. 3).

In the case of the reaction with TMSDEA, adrenochrome gave at least four major products (see Fig. 4) as shown by the GLC analysis, two of which were identified as VII and VIII. The other compounds were observed as a peak with a similar retention time to VIII and as a peak with a considerably higher retention time, which appeared to have a molecular ion of $M^{+\cdot}$ 467 (see Fig. 4). This *m/e* value suggests that this product may be the tri(trimethylsilyl) derivative VII substituted by an additional trimethylsilyl group.

It may be concluded that GLC investigations, both alone and combined with mass spectrometry, have shown that adrenolutin (II) and 5,6-dihydroxy-1-methylindole (IV) are smoothly converted to the expected trimethylsilyl derivatives by the action of HMDS–pyridine, BSA and TMSDEA. However, in addition to the tri(trimethylsilyloxy)-1-methylindole (VII) expected to be formed from adrenochrome (I), by the action of silylating agents in the presence of a base such as pyridine, significant amounts of di(trimethylsilyloxy)-1-methylindole (VIII) also appeared to be formed, suggesting that, to some extent at least, in these reactions, the silylating agents are acting as reducing agents.

REFERENCES

1 A. E. Pierce, *A Technique for Gas-Phase Analysis. Silylation of Organic Compounds*, Pierce Chem. Co., Rockford, Ill., 1968.
2 J. E. Forrest, R. A. Heacock and T. P. Forrest, *J. Pharm. Pharmacol.*, 22 (1970) 512.
3 B. LaLone and R. T. Houlihan, *Physiologist*, 14 (1971) 179.
4 R. A. Heacock, *Advan. Heterocycl. Chem.*, 5 (1965) 205.
5 R. A. Heacock and W. S. Powell, *Progr. Med. Chem.*, 9 (1972) 275.
6 J. Harley-Mason, *J. Chem. Soc.*, (1950) 1276.
7 R. A. Heacock, C. Nerenberg and A. N Payza, *Can. J. Chem.*, 36 (1958) 853.
8 R. A. Heacock and M. E. Mahon, *Can. J. Chem.*, 36 (1958) 1550.
9 G. L. Mattok and R. A. Heacock, *Can. J. Chem.*, 42 (1964) 484.