Reaction gas chromatography of basic alkoxy carbanilates with trimethylanilinium hydroxide*

[a]M. ŠTEFEK, [a]L. BENEŠ, and [b]V. KOVÁČIK

[a]*Institute of Experimental Pharmacology, Centre of Physiological Sciences, Slovak Academy of Sciences, CS-842 16 Bratislava*

[b]*Institute of Chemistry, Centre of Chemical Research, Slovak Academy of Sciences, CS-842 38 Bratislava*

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Conditions for quantitative gas-chromatographic analysis of basic alkoxy carbanilates pentacaine and heptacaine after methylation with trimethylanilinium hydroxide were examined. The flash-heater derivatization reaction is reproducible and quantitative at 280°C. The reaction products, identified as *N*-methyl derivatives of the afore-mentioned carbanilates are thermally stable and their chromatographic response is linearly proportional to the increasing concentration.

Изучены условия количественного газово-хроматографического анализа основных алкоксизамещенных карбанилатов, пентакаина и гептакаина, после их метилирования гидроокисью триметилфениламмония. Реакция, проведенная в инъекторе газового хроматографа при 280°C, проходит практически количественно, и результаты повторяются при повторных опытах. Продукты реакции были идентифицированы как *N*-метилпроизводные исследуемых карбанилатов и характеризуются тепловой стабильностью. Соответствующий им хроматографический пик растет линейно с увеличением концентрации.

Since basic alkoxy carbanilates pentacaine (*I*) and heptacaine (*II*), having local anaesthetic effect, are still undergoing clinical studies, sensitive assays for the determination of these drugs in biological material were desired. We have previously [1] reported on the gas-chromatographic behaviour of *I* and *II*; both compounds underwent a thermal degradation in the gas-chromatograph injector as a result of cleavage of the labile carbamate bond. In view of the thermal instability

* The term reaction gas chromatography (on column derivatization, flash-heater derivatization) refers to the preparation of derivatives by a simultaneous injection of the sample and derivatization reagent into the gas-chromatograph injector.

of the carbanilates, development of stable derivatives for quantitative gas-chromatographic analysis has been of considerable interest.

A sodium hydride—methyl iodide alkylation reaction of some carbanilate herbicides has been reported [2]; this relatively laborious alkylation provided thermally stable *N*-methyl derivatives. A direct methylation of substituted phenylureas [3] and *N*-phenylcarbamates [4] employing trimethylanilinium hydroxide has been described. This flash-heater derivatization reaction allowed rapid qualitative and quantitative gas-chromatographic analysis of the compounds without decomposition.

This paper concerns the methylation of *I* and *II* with trimethylanilinium hydroxide in the gas-chromatograph injector and describes conditions of gas-chromatographic analysis of the title compounds.

Bases of *I* and *II* dissolved in 0.1 M-methanolic trimethylanilinium hydroxide were derivatized by injecting 2 μl portions (concentration in a μg ml^{-1} order) into the gas chromatograph.

The products from the flash-heater reaction were identified by GC—MS analysis by comparing the retention times and mass spectra with those of reference substances *III* and *IV* as *N*-methyl derivatives of the carbanilates. The gas-chromatographic separation of thermally stable methylation products of *I* and *II* with trimethylanilinium hydroxide is shown in Fig. 1. The quantity of trimethylanilinium hydroxide required for maximum carbamate reaction was estimated employing a standard curve prepared from authentic samples *III* and *IV*.

The methylation proceeded to 98—99 % when using 2.5—15-fold molar excess of trimethylanilinium hydroxide. Fig. 2 shows the chromatographic response of *III* and *IV*. Both substances reveal a linear dependence of chromatographic response upon concentration. The lines obtained intersect the grid origin, this being indicative of no measurable degradation of the *N*-methylated derivatives on the chromatographic column. Further experiments were aimed to ascertain, whether the methylation products of *I* and *II*, obtained by derivatization with trimethylanilinium hydroxide on the column, also display a linear chromatographic response with the increasing concentration. The results, as shown in Fig. 2 by the solid blocks, clearly indicate a linear response with respect to concentration.

This methylation is reproducible, simple and proceeds quantitatively; therefore, it appears to be promising for quantitative and qualitative analysis and also for a routine screening of pentacaine, heptacaine, and related basic alkoxy carbanilates by gas chromatography.

Experimental

Pentacaine (*I*, *trans*-2-(1-pyrrolidinio)cyclohexyl-3-pentyloxycarbanilate chloride) and heptacaine (*II*, 2-(1-piperidinio)ethyl-2-heptyloxycarbanilate chloride) were syn-

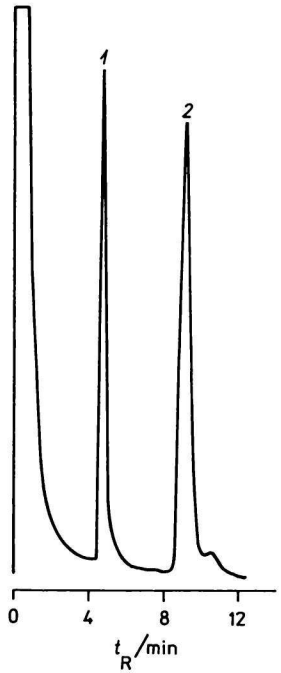

Fig. 1. Gas chromatogram of the mixture of methylation products obtained from pentacaine and heptacaine with trimethylanilinium hydroxide.
N-Methylheptacaine (*1*); *N*-methylpentacaine (*2*) (at a 10-fold molar excess of trimethylanilinium hydroxide).

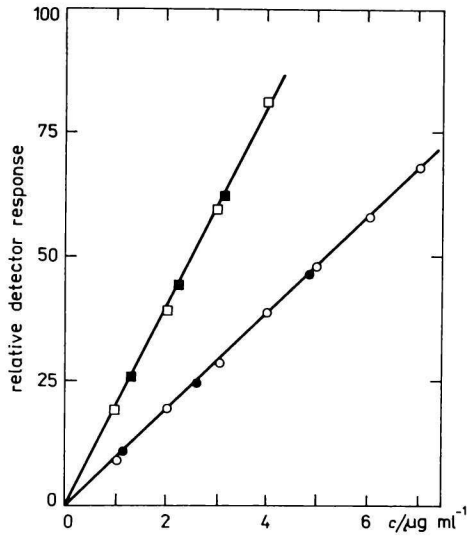

Fig. 2. Dependence of the chromatographic response on concentration of *N*-methyl derivatives of pentacaine and heptacaine.
○ *N*-Methylpentacaine; ● pentacaine + trimethylanilinium hydroxide (1:5); □ *N*-methylheptacaine; ■ heptacaine + trimethylanilinium hydroxide (1:5).

thesized by the respective authors [5, 6]. Bases of *I* and *II* liberated from aqueous solutions by addition of 2 M-Na_2CO_3 to pH 9 were extracted with ether. The derivatization reagent, 0.1 M-methanolic trimethylanilinium hydroxide was a commercial product of Serva (Heidelberg, FRG). For stock solutions, bases of *I* and *II* and their methyl derivatives *III* and *IV* were dissolved in methanol.

Gas chromatograms were recorded with a JGC-20 K (Jeol, Japan) apparatus provided with a flame ionization detector and a glass column (2 × 1000 mm), packed with 3 % OV-17 on Chromosorb W (Merck, Darmstadt, FRG), grain size 0.195—0.251 mm. Isothermal analyses were run at 260 °C column temperature and 280 °C injector temperature with nitrogen as carrier gas (0.1 MPa). The GC—MS system consisted of a gas chromatograph JGC-20 K (Jeol, Japan) and a mass spectrometer JMS-D 100 (Jeol, Japan). The gas chromatograph operated under the above-mentioned conditions, using the same column, the carrier gas being helium. The separator temperature was 270 °C, ionizing electron energy 23 eV, trap current 300 μA, ionization chamber temperature 260 °C.

Mass spectra of standard compounds were measured by a direct inlet technique at 70 eV ionizing electron energy, 220 °C ionization chamber temperature, 300 μA trap current, and

200—210 °C evaporation locus temperature. The ^{1}H-n.m.r. spectra were taken with an FX-10 FT (Jeol, Japan) spectrometer operating at 100 MHz, the i.r. spectra with a Specord 75 IR (Zeiss, Jena) apparatus.

N-Methylpentacaine (III)

Methyl iodide (710 mg; 5 mmol) and sodium hydride (72 mg; 3 mmol) were added to a stirred solution of pentacaine base (935 mg; 2.5 mmol) in dimethyl sulfoxide—benzene (8 ml, 1:1) in a moisture-protected flask at room temperature, until the evolution of gases ceased (*ca.* 1 h). Hexane (10 ml) was added, the mixture shaken for a short time (30 s), then water (10 ml) was dropwise added to decompose the excess of hydride, and finally the mixture was repeatedly shaken for 30 s. The oily product separated at the bottom was taken off, washed with ether (3 × 5 ml), and purified by preparative thin-layer chromatography using silica gel plates and ethanol—acetone—benzene—26 % ammonia (1:8:10:1) as an eluent. Purity of the product was checked by gas chromatography.

^{1}H-NMR ($CDCl_3$, δ/p.p.m.): 0.93 (t; —C—CH_3), 1.20—1.95 (m; —CH_2—), 2.52—2.70 (m; —N—CH_2—), 3.30 (s; —N(CH_3)CO—), 3.95 (t; Ar—O—CH_2—), 6.76—7.40 (m; aromatic H). IR ($CHCl_3$; cm^{-1}): 3030 ν(C—H)$_{arom}$; 2945, 2860 ν(C—H); 1705 ν(C=O); 1600, 1495 ν(C=C)$_{arom}$; 1230 ν(C—O—C); 1150 (rocking NCH_3); 1050 ν(C—O—C); 765 γ(C—H)$_{arom}$. MS: *m/z* (%) 388 (0.25), 318 (0.08), 317 (0.05), 220 (2.6), 193 (3.4), 168 (10.8), 152 (16.8), 151 (100.0), 150 (10.3), 136 (9.5), 124 (2.9), 123 (54.0), 122 (6.5), 111 (3.7), 110 (48.7), 108 (3.2), 97 (6.3), 96 (3.5), 83 (4.7), 80 (4.6), 69 (15.3), 55 (4.7), 43 (11.0).

N-Methylheptacaine (IV)

The compound was synthesized by methylation of heptacaine base with methyl iodide—sodium hydride similarly as described with *III*.

^{1}H-NMR ($CDCl_3$, δ/p.p.m.): 0.90 (t; —C—CH_3), 1.33—1.68 (m; —CH_2—), 2.63—3.13 (m; —N—CH_2—), 318 (s; —N(CH_3)CO—), 3.92 (t; Ar—O—CH_2—), 4.48 (t; —CO—O—CH_2—), 6.91—7.20 (m; aromatic H). IR ($CHCl_3$; cm^{-1}): 3035 ν(C—H)$_{arom}$; 2945, 2860 ν(C—H); 1710 ν(C=O); 1600, 1505 ν(C=C)$_{arom}$; 1240 ν(C—O—C); 1155 (rocking NCH_3); 1045 ν(C—O—C); 745 γ(C—H)$_{arom}$. MS: *m/z* (%) 376 (0.2), 292 (0.4), 221 (5.6), 124 (2.4), 123 (20.4), 122 (9.3), 112 (10.2), 111 (100.0), 110 (5.2), 109 (7.4), 108 (1.8), 105 (2.6), 99 (4.1), 98 (55.6), 97 (1.8), 96 (9.3), 84 (2.4), 83 (2.2), 70 (2.4), 69 (3.0), 57 (7.6), 56 (2.8), 55 (5.0), 43 (10.4).

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