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Note

Heat-induced conversion of N-alkylaminobenzophenones into aminobenzophenones

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Nowadays several methods are available for the analysis of benzodiazepines, a class of medicines known to influence driving skills and dominating all other medicaments found in cases of "driving under the influence"[1,2]. Important practical methods are: thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), radioimmunoassay (RIA) and enzyme-multiplied immunoassay technique (EMIT). Among these, TLC of the acid hydrolysis products of the benzodiazepines (the benzophenones) remains of considerable interest: results can be obtained in a reasonably short time and sensitivity is excellent with a detection limit of 10 ng per spot with the Bratton–Marshall[3] reaction. In this reaction the benzophenone is diazotized and then coupled with a suitable reagent, *e.g.* N-naphthylethylenediamine, to form a brightly coloured azo dye. However, only primary aminobenzophenones can be detected in this way. But several benzodiazepines, *e.g.* camazepam, diazepam, flunitrazepam, flurazepam, ketazolam, lormetazepam, prazepam and temazepam give rise to N-alkylaminobenzophenones in acid hydrolysis. The Bratton–Marshall reaction is not applicable then. In order to improve detection sensitivity, when analysing the aforementioned benzodiazepines, Schütz *et al.*[4,5] advocated photolytic desalkylation of the N-alkylated aminobenzophenones by treatment of the benzophenones with UV light. In this way all N-alkylated aminobenzophenones were at least partially converted into the corresponding aminobenzophenones.

In our previous work[6] on additional products in the hydrochloric acid hydrolysis of some benzodiazepines, we noticed the occurrence of small amounts of N-desalkylated aminobenzophenones and this prompted us to study the influence of heat on N-alkylated aminobenzophenones, the more so as a substantial increase of temperature of the chromoplate was observed in the photolytic desalkylation procedure.

In this paper we report quantitative results about the desalkylation on the chromoplate of some N-alkylated aminobenzophenones, as a function of temperature and of heating time.

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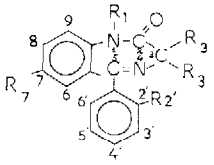
TABLE I

STRUCTURAL FORMULAE OF BENZODIAZEPINES



Benzodiazepine	R_1	R_3	R_7	$R_{2'}$
I Diazepam	CH_3	H, H	Cl	H
II Flunitrazepam	CH_3	H, H	NO_2	F
III Flurazepam	$CH_2CH_2N(C_2H_5)_2$	H, H	Cl	F
IV Lorazepam	H	H, OH	Cl	Cl
V Lormetazepam	CH_3	H, OH	Cl	Cl
VI Oxazepam	H	H, OH	Cl	H
VII Prazepam	CH_2— 	H, H	Cl	H

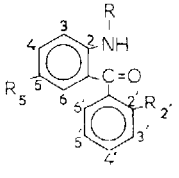
MATERIALS AND METHODS

Diazepam (I), flunitrazepam (II), flurazepam (III), lorazepam (IV), lormetazepam (V), oxazepam (VI) and prazepam (VII), respectively, were obtained by extraction of Valium® (Roche), Rohypnol® (Roche), Dalmadorm® (Roche), Temesta® (Wyeth), Loramet® (Wyeth), Seresta® (Wyeth) and Reapam® (Gödecke) tablets. (For structures of I–VII see Table I.)

Sulphuric acid hydrolysis of the benzodiazepines (I–VII) produced the corresponding benzophenones (VIII–XIV). The benzophenones were purified by a TLC procedure using toluene chloroform (9:1) as eluent. Thermal decomposition of 2-

TABLE II

STRUCTURAL FORMULAE OF BENZOPHENONES



Benzophenone	R	R_5	$R_{2'}$
VIII 2-Methylamino-5-chlorobenzophenone (MACB)	CH_3	Cl	H
IX 2-Methylamino-5-nitro-2′-fluorobenzophenone	CH_3	NO_2	F
X 2-(2-Diethylaminoethylamino)-5-chloro-2′-fluorobenzophenone	$CH_2CH_2N(C_2H_5)_2$	Cl	F
XI 2-Amino-2′,5-dichlorobenzophenone	H	Cl	Cl
XII 2-Methylamino-2′,5-dichlorobenzophenone	CH_3	Cl	Cl
XIII 2-Amino-5-chlorobenzophenone (ACB)	H	Cl	H
XIV 2-Cyclopropylmethylamino-5-chlorobenzophenone	CH_2— 	Cl	H
XV 2-Amino-5-chloro-2′-fluorobenzophenone	H	Cl	F
XVI 2-Amino-5-nitro-2′-fluorobenzophenone	H	NO_2	F

methylamino-5-chloro-2′-fluorobenzophenone, using the same TLC purification procedure, produced 2-amino-5-chloro-2′-fluorobenzophenone (XV). Details of the isolation of 2-amino-5-nitro-2′-fluorobenzophenone (XVI) are reported in ref. 6. The structures of the benzophenones were confirmed by mass spectrometry (MS). (For structures of VIII–XVI see Table I.)

Thermal desalkylation of N-alkylaminobenzophenones

The benzophenones were dissolved in chloroform (Merck 2432 "zur Rückstandsanalyse"). Solutions of *ca.* 0.1 mg/ml and *ca.* 0.01 mg/ml were used, and 100 μl and 500 μl, respectively, of these solutions were spotted (in 25 and 125 spots respectively) on a TLC plate (Merck 5724, Kieselgel 60) of 0.25 mm layer thickness. Therapeutic concentration ranges were covered in this way (*ca.* 40–*ca.* 400 ng a spot). The plates were heated in a drying box for 1 h at 100, 120, 140, 160, 180 and 200°C. Experiments were also done at a constant temperature of 160°C, for various times (1, 2 and 4 h).

Gas chromatography

After heating, the bands on the plates were scratched off and the silica gel material extracted three times with acetone. The acetone was evaporated at a slightly elevated temperature under a gentle stream of nitrogen. Then the internal standard *n*-tetracosane in chloroform was added, and the amounts of both N-alkylated and

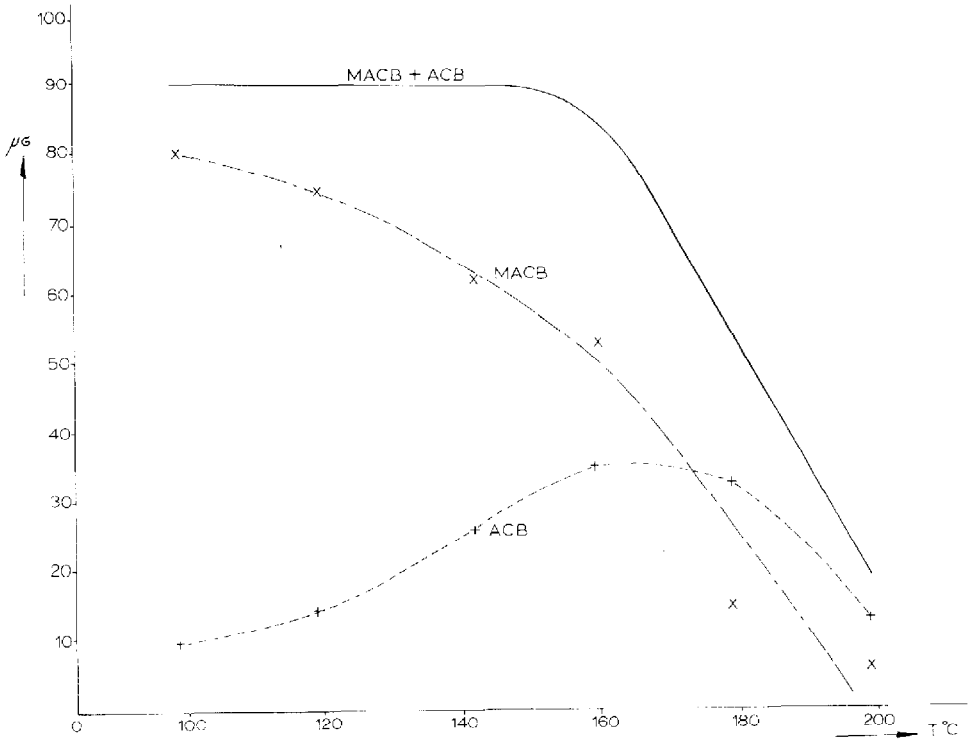


Fig. 1. Thermal decomposition of 2-methylamino-5-chlorobenzophenone (MACB) to 2-amino-5-chloro benzophenone (ACB); *ca.* 100 μg of MACB is spotted.

TABLE III

HEAT-INDUCED DECOMPOSITION OF N-ALKYLAMINOBENZOPHENONES

Heating time 1 h. Chromoplate: Kieselgel 60, layer thickness 0.25 mm. A = Percentage of 2-amino-5-chlorobenzophenone (XIII) found, relative to the amount originally spotted. B = Percentage of 2-amino-5-chloro-2'-fluorobenzophenone (XV) found, relative to the amount originally spotted. C = Percentage of 2-amino-2,5'-dichlorobenzophenone (XI) found, relative to the amount originally spotted. D = Percentage of 2-amino-5-chlorobenzophenone (XIII) found, relative to the amount originally spotted.

Temperature (°C)	*Decomposition of 2-methylamino-5-chlorobenzophenone (IX)*		*Decomposition of 2-(2-diethylaminoethylamino)-5-chloro-2'-fluorobenzophenone (X)*		*Decomposition of 2-methylamino-2',5-dichlorobenzophenone (XII)*		*Decomposition of 2-cyclopropylmethylamino-5-chlorobenzophenone (XIV)*	
	*A**	*A***	*B**	*B***	*C**	*C***	*D**	*D***
100	6.2	9.2	1.4	1.5	3.3	< 1.0	10.0	8.1
120	13.9	10.8	2.3	2.9	4.1	2.1	16.3	13.0
140	22.0	13.8	3.1	3.9	8.3	4.1	20.6	13.7
160	27.9	18.8	4.0	5.8	14.5	6.8	24.8	14.5
180	28.6	6.9	2.8	1.0	16.4	13.0	20.0	17.2
200	13.5	6.0	< 1.0	< 1.0	16.6	16.0	8.2	9.8

* Amount spotted *ca.* 10 μg.
** Amount spotted *ca.* 1 μg.

N-desalkylated aminobenzophenones were measured by GC. A Perkin-Elmer Sigma 3 equipped with a Hewlett-Packard 3390A integrator was used for this purpose. Chromatographic conditions were: column, 25 m × 0.32 mm I.D. fused silica, wall-coated open tubular (WCOT), Cp™ Sil 5 CB; carrier gas, nitrogen; flow-rate, *ca.* 2 ml/min; oven temperature, 220°C; injector and detector temperature, 275°C.

Spectroscopy

Electron impact spectra (70 eV) of the benzophenones were taken for confirmation purposes on a Finnigan MAT 212 GC–MS combination, coupled to a MAT Spectro System 100 MS computer. Low-resolution mode ($\Delta M/M \approx 1000$) was used. Ion source and GC MS interface temperatures were 300°C and 250°C, respectively. Accelerating voltage was 3 kV, and an ionization current of 0.5 mA was used. Chromatographic conditions were: column, WCOT, Cp™ Sil 5 on fused silica, 25 m × 0.32 mm I.D.; oven temperature, 200°C; carrier gas, helium; flow-rate, *ca.* 2.5 ml/min.

RESULTS AND DISCUSSION

Fig. 1 shows results of experiments at a level of *ca.* 100 μg of benzophenone "spotted" for the desalkylation of 2-methylamino-5-chlorobenzophenone (MACB). After heating for 1 h at different temperatures, mixtures of 2-amino-5-chlorobenzophenone (ACB) and MACB were found. Recovery is perfect, if the temperature is lower than 160°C. Increasing the temperature above 160°C causes a considerable decrease in the amounts of both ACB and MACB. The same results are also found at levels of 10 μg and 1 μg of MACB "spotted". A similar picture was found for the other convertible N-alkylated aminobenzophenones listed in Table II, although the temperature of imperfect recovery varied somewhat from 160°C. In our opinion this could be ascribed to evaporation of the benzophenones.

Table III shows the results of desalkylation at various temperatures at a constant heating time of 1 h for 10 μg and 1 μg spots of the following benzophenones: (i) 2-methylamino-5-chlorobenzophenone; (ii) 2-(2-diethylaminoethylamino)-5-chloro-2′-fluorobenzophenone; (iii) 2-methylamino-2′,5-dichlorobenzophenone; (iv) 2-cyclopropylmethylamino-5-chlorobenzophenone. Under these circumstances the desalkylation of 2-methylamino-5-nitro-2′-fluorobenzophenone appeared to be negligible. With all other N-alkylated aminobenzophenones the temperature of 160°C was found to be optimal.

Table IV shows the results of desalkylation at a constant temperature for various times for the same benzophenones. A heating time of 2 h appeared to be the best.

CONCLUSION

From the results in Table III and IV it can be concluded that with the exception of the benzophenone of flunitrazepam, the benzophenones of camazepam, diazepam, flurazepam, ketazolam, lormetazepam, prazepam and temazepam can be converted by heat into primary aminobenzophenones suitable for diazotization, so that the detection limits of the N-substituted aminobenzophenones can be improved. A detection limit of 10–50 times lower can be reached compared with the detection limit

TABLE IV

HEAT-INDUCED DECOMPOSITION OF N-ALKYLAMINOBENZOPHENONES

Temperature, 160°C. Chromoplate: Kieselgel 60, layer thickness 0.25 mm. A = Percentage of 2-amino-5-chlorobenzophenone (XIII) found, relative to the amount originally spotted. B = Percentage of 2-amino-5-chloro-2'-fluorobenzophenone (XV) found, relative to the amount originally spotted. C = Percentage of 2-amino-2,5'-dichlorobenzophenone (XI) found, relative to the amount originally spotted. D = Percentage of 2-amino-5-chlorobenzophenone (XIII) found, relative to the amount originally spotted.

Time (h)	*Decomposition of 2-methylamino-5-chlorobenzophenone (IX)*		*Decomposition of 2-(2-diethylaminoethylamino)-5-chloro-2'-fluorobenzophenone (X)*		*Decomposition of 2-methylamino-2',5-dichlorobenzophenone (XII)*		*Decomposition of 2-cyclopropylmethylamino-5-chlorobenzophenone (XIV)*	
	*A**	*A***	*B**	*B***	*C**	*C***	*D**	*D***
1	27.9	18.8	4.0	5.8	14.5	6.8	24.8	14.5
2	35.0	26.7	3.7	6.7	22.7	20.9	24.0	29.3
4	36.4	15.8	2.0	1.1	27.1	24.2	18.7	21.7

* Amount spotted *ca.* 10 μg.
** Amount spotted *ca.* 1 μg.

for N-alkylaminobenzophenones by UV light of wavelength 254 nm on plates with an added fluorescence indicator.

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REFERENCES

1 R. Th. Twente, A. B. E. Theeuwen and A. M. A. Verweij, *Pharm. Weekbl.*, 110 (1975) 496.
2 W. Neuteboom and P. G. M. Zweipfenning, *T. Alc. Drugs*, 9 (1983) 59.
3 A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, 128 (1939) 537.
4 H. Schütz, *Benzodiazepines*, Springer, Berlin, Heidelberg, New York, 1982.
5 H. Schütz, H. Fitz and S. Suphachearabhan, *Z. Anal. Chem.*, 314 (1983) 44.
6 M. M. A. de Bruyne, A. Sinnema and A. M. A. Verweij, *J. Forensic Sci.*, submitted for publication.