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RELATIVE ELECTRON CAPTURE RESPONSE OF THE 2-CHLOROETHYL DERIVATIVES OF SOME BARBITURIC ACIDS AND ANTICONVULSANT DRUGS

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SUMMARY

Data are given for the relative electron capture response of the 2-chloroethyl derivatives of ten barbituric acids and five common anticonvulsant drugs. Results indicate that these new derivatives significantly improve the detection limits of the barbituric acids but lead to no advantages for the anticonvulsant compounds. Structural features in the two classes of compounds are believed to explain the differences in the sensitivity of detection. Linear calibration plots exist for the concentration ranges 0.1–1.0 and 1.0–10.0 μg/ml of amobarbital.

INTRODUCTION

Whereas barbiturate analyses by gas chromatography have been based almost exclusively on flame ionisation detection, study of the electron capture detection of barbiturates has been confined to some free barbituric acids[1], and more recently, their pentafluorobenzyl derivatives[2,3]. Yet, analytical methods based on the electron capture of the 2-chloroethyl esters of short-chain fatty acids[4] were investigated many years ago. More recently, analytical methods utilizing the 2-chloroethylation of the herbicides 2-methyl-4-chlorophenoxyacetic acid[5] and 2,4-dichlorophenoxyacetic acid[6], and bis(4-chlorophenyl) acetic acid, a metabolite[7] of DDT, have been reported.

Following the synthesis[8,9] of the 2-chloroethyl derivatives as a prerequisite to the quantification of barbiturates and some common anticonvulsant drugs, we report here the electron capture response of the 2-chloroethyl derivatives of ten barbituric acids and several ethyl and methyl derivatives, and their detection limits. Included also are the detection limits of the 2-chloroethyl derivatives of a number of common anticonvulsant drugs. All the derivatives were prepared by reaction of the barbituric acid or anticonvulsant drug with the appropriate dialkyl sulphate in a mildly alkaline medium rather than with the Claisen-type reaction employed for the pentafluorobenzyl[2,3], benzyl, allyl and methoxymethyl derivatives[10,11].

TABLE I

GAS CHROMATOGRAPHIC PROPERTIES OF BARBITURIC ACIDS AND THEIR N-ALKYLATED DERIVATIVES

R_1 and R_2 are substituents at C-5 of the pyrimidine ring.

Barbiturate	*Detection limit (ng)*						*Column temp. (°C)*	*Retention time (min)*
	Free acid	*Dimethyl*	*Monoethyl*	*Diethyl*	*Monochloroethyl*	*Bischloroethyl*		
Amobarbital	8.5						200	1.0
(R_1 = ethyl,		111					155	1.8
R_2 = 3-methylbutyl				136			155	2.4
						0.16	225	1.1
Pentobarbital	4.0						200	1.1
(R_1 = ethyl,		20					155	1.9
R_2 = 1-methylbutyl)				26			155	2.7
						0.10	225	1.2
Phenobarbital								
(R_1 = ethyl,		28					185	1.7
R_2 = phenyl)				14			185	2.0
						0.14	235	1.6
Mephobarbital	9.3						200	1.9
(R_1 = ethyl,			13				185	1.8
R_2 = phenyl)					0.36		205	2.1
Barbital								
(R_1 = ethyl,				115			155	1.1
R_2 = ethyl)						0.12	205	1.3
Secobarbital	0.07						200	1.9
(R_1 = 1-methylbutyl,				0.88			155	3.6
R_2 = prop-2-enyl)						0.12	225	1.3
Secbutobarbital								
(R_1 = ethyl,						0.07	205	1.9
R_2 = 1-methylpropyl)								
Butobarbital								
(R_1 = ethyl,						0.12	205	2.0
R_2 = butyl)								

EXPERIMENTAL

A Hewlett-Packard 5750 gas chromatograph was used with a 2-mC ^{63}Ni electron capture detector (ECD) operated at 240°. A coiled borosilicate glass column (1.63 m × 6.4 mm O.D.) packed with 3% SE-30 (w/w) on Chromosorb 750 (100–120 mesh) and re-silanized with hexamethyldisilazane prior to use, was employed throughout this work. The carrier gas [argon–methane (95:5)] flow-rate was maintained at 72–75 ml/min except for the 5,5'-diphenylhydantoin derivatives (136 ml/min). A pulsed voltage was applied to the detector (amplitude 30 V, period 50 μsec, width 0.75 μsec).

The ECD response was determined by injection of known quantities (1.5–2.5 μl) of pure derivatives[8,9] dissolved in hexane or ethyl acetate. Minimum detectable quantities in nanograms, based on a peak height signal of three times the background noise level, were determined by duplicate injections of the individual compounds. Retention times for the derivatives were adjusted to 1–2.5 min by alteration of the column temperature. In contrast, the free barbituric acids were chromatographed isothermally at 200° after saturation of the active sites in the column with repeated injections of the free acids. This procedure was adopted in order to obtain reproducible[12–15] data for the acids and to avoid the variation[1,16,17] in retention times caused by the adsorption of submicrogram amounts of barbituric acids on the column.

RESULTS AND DISCUSSION

Detection limits for the derivatives of the barbituric acids and the anticonvulsant compounds are given in Tables I and II respectively. To facilitate comparison, the detection limits of the free-acid forms of these compounds are also included. However, interpretation of the data for the free barbituric acids is complicated by their typical chromatographic behaviour of displaying considerable tailing on the silanized, non-polar column. Because of this characteristic, the detection limits for the free barbituric acids were approximated by measuring the areas of the distorted peaks and are expressed as the quantity of acid in nanograms per unit area (here, 50 mm^2). The free acids of the anticonvulsant drugs, with the exception of ethosuximide and diphenylhydantoin, gave reasonably symmetrical peaks so that detection limits could be obtained by the measurement of peak heights. All alkylated derivatives exhibited symmetrical chromatographic peaks and showed no evidence of adsorption.

Since an adequate understanding of the mechanism of electron capture is important if further reduction of detection limits is to be achieved, attempts to explain differences in response have endeavoured to identify the structural region of a molecule associated with the electron capture process. Thus, Landowne and Lipsky[18] proposed that the carbonyl carbon atom, and not the halogenated α-carbon atom of the introduced group, was responsible for the initial electron capture in a series of cholesteryl haloacetates. Clarke *et al.*[19] made a further important distinction between O-monochloroacetyl and N-monochloroacetyl derivatives to account for the reduced sensitivity of the amine derivatives towards electron capture. In the amine derivative, delocalization of the positive charge on the carbonyl carbon atom, through a resonance mechanism involving the lone-pair of the nitrogen atom, was seen as the influence opposing the effect of the particular halogenated group. Although it was recognised

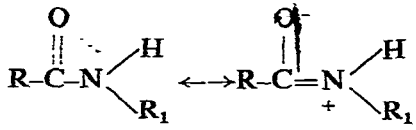
TABLE II

GAS CHROMATOGRAPHIC PROPERTIES OF ANTICONVULSANT COMPOUNDS AND THEIR N-ALKYLATED DERIVATIVES

Anticonvulsant	*Detection limit (ng)*				*Column temp. (°C)*	*Retention time (min)*
	Free acid	*Monoethyl*	*Mono-chloroethyl*	*Bis-chloroethyl*		
Peganone (3-ethyl-5-phenyl-imidazolidine-2,4-dione)	49				185	1.6
			55		185	2.6
Mephenytoin (5-ethyl-3-methyl-5-phenylimidazolidine-2,4-dione)	20				185	1.5
			51		185	3.2
Glutethimide (3-ethyl-3-phenyl-piperidine-2,6-dione)	27				185	1.8
		652			185	1.8
			86		205	2.1
Ethosuximide (3-ethyl-3-methyl-pyrrolidine-2,5-dione)	36				125	1.3
			71		155	0.9
Diphenylhydantoin (5,5-diphenylimida-zolidine-2,4-dione)		111*			235	1.2
			20**		235	1.5
				1.3	235	2.2

* Ethylated at N-3.
** Chloroethylated at N-3.

that electron capture could possibly occur in a polyhalogenated chain, such as in N-pentafluoropropionamide and N-heptafluorobutyramide derivatives of amines, the explanation was consistent even for the least sensitive N-trifluoroacetylated amine derivative because of the same counteractive mechanism. Again, a detailed study of various derivatives of primary and secondary amines by Matin and Rowland[20] supported the concept that an amide functionality, where the groups C=O and C=N were present simultaneously, provides the electron-deficient centre necessary for good ECD response. Furthermore, in the general representation:



it was shown that the greater the electron-withdrawing inductive effect of the substituent R_1, the greater was the polarizability of the carbonyl group and, consequently, its electron-capturing capacity.

Although it is a unique cyclic amide, a resonance mechanism such as that outlined above can be visualized as operating in the barbiturate nucleus also. The magnitude of the electron-deficiency of the electrophore would be influenced by the nature

of a substituent on one or both nitrogen atoms. Replacement of an imino hydrogen by an ethyl or methyl group could be expected to lead to a reduction in electron-deficiency at the nitrogen atom(s) due to an electron-releasing inductive effect of the alkyl groups. In this way, polarization of the adjacent carbonyl groups would be diminished and lead to reduced ECD response. Introduction of an electron-withdrawing group, such as a chloroethyl substituent, would reverse the effect and enhance the ECD response. The results presented in Table I lend support to this hypothesis, particularly for the derivative compounds. However, because of adsorption of the barbituric acids on the column, direct comparison of the detection limits of acids and derivatives may not be valid. Of interest too is the wide range of responses obtained for the methyl and ethyl derivatives in contrast to the chloroethylated barbiturate derivatives (with the exception of hexobarbital) which show a uniform and significantly higher ECD response falling within a relatively small range of detection limits. A similarly narrow (albeit, considerably lower) range of detection limits has been observed[2] for the pentafluorobenzyl derivatives. Both of these separate findings suggest that a limiting effect may be conferred upon the overall molecule by the electrophilic N-substituent.

As a final comment on the results in Table I, attention may be drawn to the paucity of evidence regarding the influence of the C-5 substituents. Gudzinowicz and Clark[1] suggested that the ability of a particular barbituric acid to capture electrons depends to some extent on the substituent and decreases in the order: phenyl > cyclohex-1-enyl > alkyl. Although the narrow range of detection limits does restrict interpretation of the results for the chloroethyl derivatives, taking the five barbituric acids differing only in one of the C-5 substituents (that is, amobarbital, barbital, cyclobarbital, pentobarbital and phenobarbital) and their methylated and ethylated derivatives, the response decreases in the order: cyclohex-1-enyl > phenyl, secondary alkyl > primary alkyl. The effect of the different C-5 substituents on the overall ECD response of the molecule cannot be explained however, and other factors[21] such as the stability of the resultant negative ion or electron capture by the products of a dissociative step may influence the ultimate ECD response. The unusually high response for secobarbital, and to a lesser extent its ethyl derivative, is probably due to the prop-2-enyl substituent, in agreement with the finding[22] that a doubly-bonded alkyl moiety makes a substantial contribution to the electron-capturing capability of a molecule.

When contrasted with the chloroethylated barbituric acids, only a weak ECD response was observed for the chloroethyl derivatives of the anticonvulsant compounds. This result is attributed to the different molecular structure comprising only two polarizable carbonyl groups, and with the exception of diphenylhydantoin, only one nitrogen available for alkylation. The polarizability of the carbonyl groups in these molecules is thus limited, in comparison with the barbiturates, by the reduced number of possible resonance forms. Moderate ECD response is seen only in the bis-(chloroethyl)derivative of diphenylhydantoin. It appears that the electron-releasing inductive effect of the ethyl group again contributes to the low response of the ethyl derivative of glutethimide relative to that of the parent compound and that of the N-3 ethylated diphenylhydantoin compared with its chloroethylated analogue.

The value of the chloroethyl derivatives can be seen in the linearity of the ECD response for the bis(chloroethyl)amobarbital derivative. A linear response was found for the range 0.1–1.0 μg/ml at a pulse period of 50 μsec (see Fig. 1), and has been demonstrated for the range 1.0–10.0 μg/ml when the pulse period was reduced to

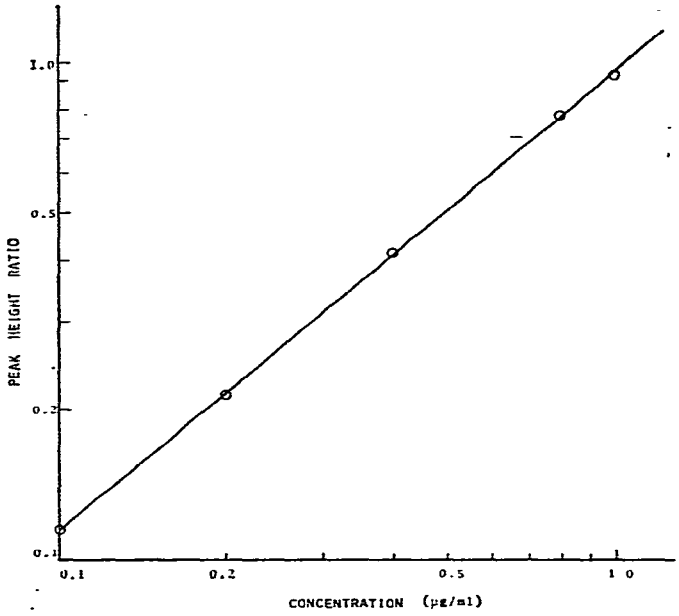


Fig. 1. Calibration curve for bis(chloroethyl)amobarbital using bis(chloroethyl)cyclobarbital (at 1.0 μg/ml) as internal standard. Each point on the curve is a mean value from two determinations.

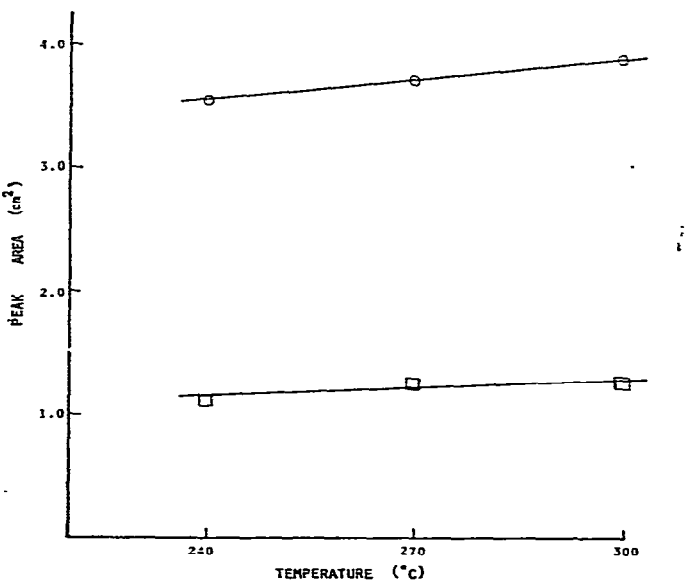


Fig. 2. The relationship between ECD response and detector temperature for bis(chloroethyl)secobarbital (○) and bis(chloroethyl)amobarbital (□). Each point on the curves is a mean value from two determinations at concentrations of 1 μg/ml. Column temperature, 225°; electrometer sensitivity, 128 × 1.

5 μsec. Although the electron affinity of many compounds has been shown[23] to be strongly dependent on the detector cell temperature, little variation in response is evident for the chloroethyl derivatives of amobarbital and secobarbital (see Fig. 2) when the cell temperature was varied between 240° and 300°.

It may be concluded that the 2-chloroethylation of barbituric acids leads to a significant improvement in their sensitivity to electron capture gas chromatography. However, with the exception of diphenylhydantoin, chloroethylation of the anticonvulsant compounds considered here confers no enhancement upon their response so that for these compounds the main advantage in the derivatives lies in possible qualitative applications. The development of a quantitative analytical scheme for the barbiturates is being explored. Currently, the near-complete conversion of the acid form of amobarbital at the 0.5 μg/ml level has been successful.

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