CHROM. 4431

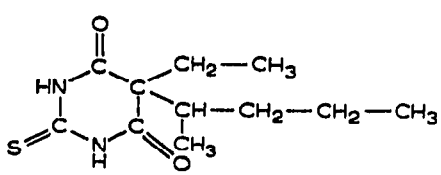
REACTION THIN-LAYER CHROMATOGRAPHY OF THIOBARBITURATES AND BARBITURATES AFTER BROMINATION AT THE STARTING POINT

ROKUS A. DE ZEEUW AND JAAP WIJSBEEK
Laboratory for Pharmaceutical and Analytical Chemistry, State University, Antonius Deusinglaan 2, Groningen (The Netherlands)

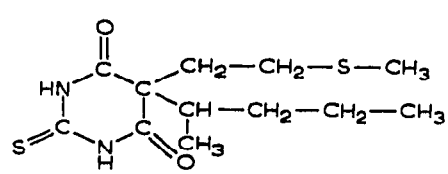
SUMMARY

Bromination of the spots at the starting point has been applied in the identification of closely related thiobarbiturates and barbiturates. The method proved to be very suitable to attack unsaturated derivatives and other reactive components, which can thus be distinguished from close-lying saturated and less reactive compounds. Oxidation reactions are mainly involved, taking place very rapidly. The adsorbent was shown to play an important role because addition of bromine to the substances dissolved in an organic solvent did not yield the same products as after bromination on the plate. The method seems to be likewise applicable to other classes of compounds.

During previous investigations on the separation and identification of thiobarbiturates[1], it was observed that thiopental and methitural could hardly be separated by normal thin-layer chromatography (TLC) procedures, in spite of the relatively large difference in one of the substituents at C_5.



Thiopental



Methitural

After testing a variety of solvents, separation could only be achieved by means of chloroform–methanol (95:5) on Silica Gel GF_{254} as adsorbent, the hR_F values of methitural and thiopental being 65 and 58, respectively.

It was felt, however, that a more distinct separation would enhance the identification possibilities, and we therefore started to carry out bromination of the substances directly at the starting points, then followed by development. 5 μl of a 2% (v/v) solution of bromine in chloroform was applied to react with about 10 μg of substance. Reaction takes place immediately. Development was performed on Silica Gel GF_{254} with chloroform–ether (75:25) in unsaturated chambers, the solvent front being 15 cm from the starting points.

With this method methitural and thiopental could be clearly distinguished. Thiopental is converted into pentobarbital, 5-ethyl-5-(1-methylbutyl)barbituric acid, which shows an hR_F value of about 50, whereas the oxidation products of methitural remain at the starting point (see Table I). It may be expected that, in methitural, conversion to a normal malonylurea ring will occur together with an oxidation of the methylthioethyl substituent at C_5, yielding an alcohol or a carboxylic acid. According to MAYNERT AND WASHBURN[2], dealkylation may occur as well, but no conclusive evidence is yet available.

We also investigated the reaction chromatography of other barbiturates after treatment with bromine, particularly with respect to those cases in which an unsaturated and a saturated barbituric acid derivative showed similar R_F values. Again the method proved to be valuable. Unsaturated derivatives, *e.g.* with allyl, vinyl or cyclohexenyl substituents, are rapidly converted into products showing a distinctly lower R_F value than the parent compound. It seems likely that oxidation of the reactive substituent occurred, *e.g.* yielding an alcohol, a ketone or a carboxylic acid, because these products will give rise to a lower R_F value. The malonylurea ring is not attacked because the reaction products are still capable of giving a strong Parri reaction after spraying with cobalt nitrate in chloroform followed by exposure to ammonia vapours; in this case, the reaction is given by the ring structure only. Addition of bromine at the double bond may take place as a first step in the oxidative attack, but this will be immediately followed by conversion to other products. This can be concluded from the fact that bromine-addition products will give spots with a slightly higher R_F value than the parent compound; such spots are not observed, however. A few examples in which bromination at the starting points was applied successfully can be seen in Table I.

TABLE I

hR_F VALUES OF THIOBARBITURATES AND BARBITURATES WITH AND WITHOUT BROMINATION AT THE STARTING POINTS

Common name	*Formula*	hR_F	
		Untreated	*Treated*
Methitural	5-(1-methylbutyl)-5-(2-methylthioethyl)-2-thiobarbituric acid	96	0
Thiopental	5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid	96	50
Aprobarbital	5-allyl-5-isopropyl-barbituric acid	49	10
Butobarbital	5-butyl-5-ethyl-barbituric acid	49	49
Vinbarbital	5-ethyl-5-(1-methyl-1-butenyl)-barbituric acid	47	29

It should be noticed that the adsorbent plays an important role in the reaction. When bromide is added to a *solution* of the barbiturates, addition of bromine at the double bond is chiefly obtained, thus giving spots with a slightly higher R_F value than the parent compound. Bromination *on the plate*, however, mainly gives products with a distinctly lower R_F value than the parent component. A further advantage of the latter method is that each spot can be treated or left untreated as desired and that untreated reference compounds can be run on the same plate as well. This makes identification much easier. During bromination, however, the spots which are to be

left untreated must be protected carefully because bromine is rapidly attracted by the adsorbent and reacts immediately with the spotted components.

It should be remembered that in many cases the reactions on the plate do not go to completion and/or are chain reactions. This can be disadvantageous in some instances, but on the other hand this phenomenon may give a pattern of reaction products of specific nature, thus allowing positive identification of the unknown compound. With barbiturates, however, usually one or two reaction products were observed. The occurrence of chain reactions or incomplete reactions, of course, makes the described technique in general unsuitable for purity tests and preparative or quantitative work.

Further studies on other classes of substances have revealed that bromination at the starting points can be a rapid and versatile aid to identification procedures in various fields of analysis.

REFERENCES

1 R. A. DE ZEEUW AND J. WIJSBEEK, *Pharm. Weekblad*, 104 (1969) 901.
2 E. W. MAYNERT AND E. J. WASHBURN, *J. Am. Chem. Soc.*, 75 (1953) 1700.