Separation and Identification of Barbiturates and Some Related Compounds by Means of Gas-Liquid Chromatography

By E. BROCHMANN-HANSSEN and A. BAERHEIM SVENDSEN

Barbiturates and certain related hypnotics have been separated and identified by direct gas chromatography on a number of different stationary phases. Depending on the type and concentration of stationary phase, column temperatures ranging from about 140 to 200° were used. No evidence of decomposition of the substances was observed. The method is rapid and extremely sensitive.

RELATIVELY PURE single barbiturates can easily be identified by a number of methods, including the melting points of derivatives (1–4), infrared spectrophotometry (5–9), X-ray diffraction (10–15), and crystallography (16–20). When several barbiturates are present in a mixture, or when the material is contaminated with decomposition products and/or substances of biological origin, it is usually necessary to perform a separation prior to the identification process. Fractional extraction (21) and countercurrent distribution (22) have been used with some degree of success. Column partition chromatography (23) and adsorption chromatography (24) have given good results for specific problems of limited complexity. However, paper chromatographic procedures with a variety of solvent systems have proved the most satisfactory for more difficult separations (25–29). Yet, there are several combinations of barbiturates which have not been adequately resolved by this method. Furthermore, paper chromatography is somewhat time-consuming and fairly insensitive with respect to barbiturates.

In recent years, gas-liquid chromatography has proved to be an exceptionally valuable tool for qualitative and quantitative analysis of many types of compounds. Development of highly sensitive detectors and the use of columns containing low concentrations of the stationary phase have made the gas chromatographic method applicable to substances of very low volatility.

Attempts to identify barbiturates by gas chromatography have been reported by Janák (30, 31). He pyrolyzed the sodium salts and gas chromatographed the pyrolysis products. This paper describes a method for direct gas chromatographic separation and identification of barbituric acid derivatives and some related substances (32).

EXPERIMENTAL

A Barber-Colman model 15 gas chromatograph equipped with an argon ionization detector was used for the experimental work. The columns were glass U-tubes, 6 feet in length, and having an inner diameter of 3 mm. The solid support was Chromosorb W, 60–80 mesh, washed with concentrated

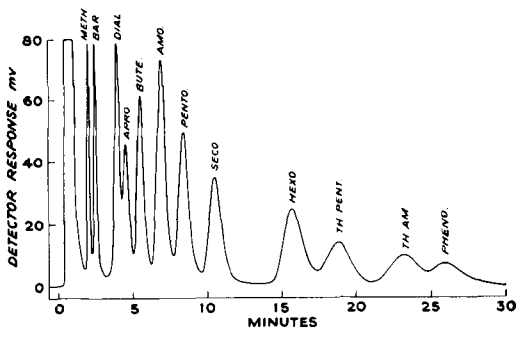

Fig. 1.—Gas chromatogram of 12 barbiturates on Apiezon L. The experimental conditions and the names of the barbiturates refer to Table I.

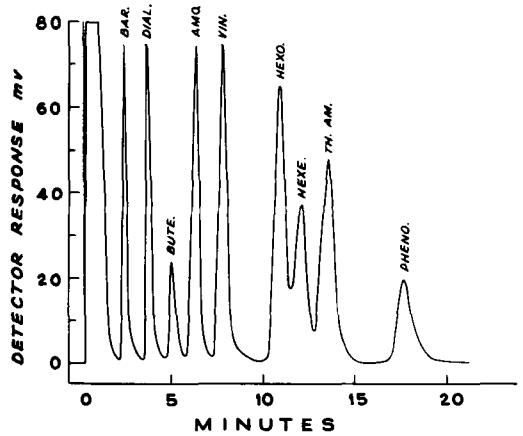

Fig. 2.—Gas chromatogram of nine barbiturates on SE-30. The experimental conditions and the names of the barbiturates refer to Table I.

Received July 25, 1961, from the University of California School of Pharmacy, Medical Center, San Francisco 22.
Accepted for publication September 18, 1961.
This work was supported by a research grant (M-3487) from the National Institutes of Health.

After this paper was submitted for publication, a paper entitled "Separation and Identification of Barbiturates by Gas Chromatography," by K. D. Parker and P. L. Kirk appeared in *Anal. Chem.*, 33, 1378(1961).

TABLE I.—RELATIVE RETENTION TIMES OF BARBITURATES AND RELATED COMPOUNDS ON COLUMNS WITH VARYING POLARITY

Compound	Apiezon L.	SE-30[a]	SE-52[b]	NGSE[c]	PEG 4000[d]	QF-1-0065[e]
Allylisopropylacetylurea	0.40	0.45	0.43	...	...	...
Amobarbital	0.82	0.92	0.90	0.92	0.92	0.96
Aprobarbital	0.55	0.56	0.56	0.76	0.85	0.63
Barbital	0.29	0.32	0.33	0.50	0.53	0.47
5(2-Br. allyl)-5-isopropyl. b.a.	1.77	1.91	1.92	2.62	4.30	1.80
Butethal	0.65	0.71	0.70	0.86	0.84	0.80
Cyclobarbital	3.42	3.03	3.02	2.72	4.32	2.50
Cyclopal	2.06	1.82	1.86	2.03	3.14	1.47
Diallylbarb. acid	0.46	0.52	0.52	0.78	0.97	0.57
Glutethimide	1.98	1.36	1.46	0.86	0.53	1.67
Hexethal	2.15	1.94	1.84	1.51	1.66	1.51
Hexobarbital	1.88	1.61	1.65	0.89	0.62	1.54
Itobarbital	0.68	0.71	0.70	0.89	0.91	0.74
Mephobarbital	2.30	1.91	1.97	1.25	1.04	1.67
Metharbital	0.24	0.23	0.24	0.21	0.11	0.34
Pentobarbital	1.00	1.00	1.00	1.00	1.00	1.00
Phenobarbital	3.10	2.88	2.95	4.14	8.78	2.50
Probarbital	0.44	0.42	0.44	0.63	0.65	0.57
Sec. Butyl Br. allyl. b.a.	3.39	2.98	3.02	3.41	5.54	2.41
Secobarbital	1.26	1.29	1.27	1.17	1.38	1.09
Thiamylal	2.82	2.05	2.05	1.63	1.41	1.09
Thiopental	2.21	1.59	1.60	1.32	1.08	1.01
Vinbarbital	0.99	1.17	1.16	1.13	1.45	1.12
Temperature, ° C.	150	137	144	200	170	165
Inlet pressure, p.s.i.	20	25	20	20	20	16
Outlet flow rate, ml./min.	37.5	68	47	58	40	25
Support, mesh	60–80	60–80	60–80	60–80	60–80	60–80
Liquid phase, w/w %	1.4	1.2	1.4	1.7	0.6	2.8
Pentobarbital time, min.	8.4	6.6	6.3	6.4	7.4	7.6

[a] Methyl silicone polymer, General Electric Co. [b] Phenyl silicone polymer, General Electric Co. [c] Neopentyl glycol sebacate. [d] Polyethylene glycol 4000. [e] Fluorosilicone fluid QF-1-0065, 10,000 cs., Dow Corning Corp.

hydrochloric acid and methanolic potassium hydroxide, and treated with hexamethyldisilazane to reduce adsorptive effects (33). The stationary phase was applied by means of a solution in toluene or acetone as described by Horning, *et al.* (34).

The barbiturates were introduced with a Hamilton microsyringe as 1.0 μl. of a 0.5 to 1.0% solution in acetone. Sodium salts of barbiturates were converted to free acids by means of dichloroacetic acid in acetone (35).

The results for 21 barbiturates and two related compounds, glutethimide and allylisopropylacetylurea in several stationary phases ranging from nonpolar to very polar materials are shown in Table I. Pentobarbital was used as standard for calculation of the relative retention times. The temperature and pressure were adjusted so as to produce nearly the same retention time for pentobarbital on all columns. This required a temperature of 200° for neopentyl glycol sebacate, while about 140° was sufficient for the silicone rubber materials. Most substances investigated gave sharp single component peaks consistent with absence of decomposition. In a few instances, one or two small additional peaks were observed due, presumably, to impurities in the samples available. The efficiency of the gas chromatographic method for separation of mixtures of barbiturates is illustrated in Figs. 1–4. Amobarbital and pentobarbital which are difficult to separate by paper chromatography, are separated nicely on an Apiezon L column (Fig. 1). Figure 4 shows a gas chromatogram obtained with nonlinear temperature programming from 150 to 225° on a polyester column. Temperature programming extends the range of application, shortens the time, and makes the component peaks sharper. Not all

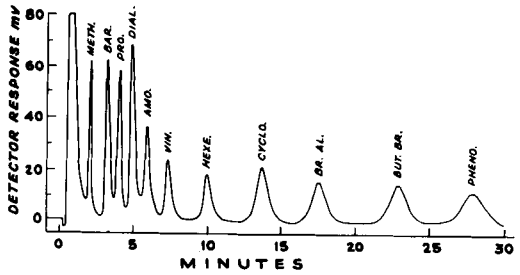

Fig. 3.—Gas chromatographic separation of 11 barbiturates on NGSE. The experimental conditions and the names of the barbiturates refer to Table I. CYCLO = cyclopal.

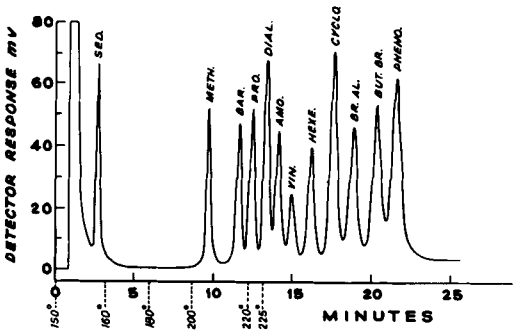

Fig. 4.—Gas chromatogram of 11 barbiturates and allylisopropylacetylurea (Sedormid) on NGSE obtained by nonlinear temperature programming.

substances examined could be separated on the same column. However, by using two columns, one with a nonpolar stationary phase such as Apiezon and one with a moderately polar polyester phase, a large number of possible combinations could easily be separated. The components were identified on the basis of their relative retention times (Table I). This method was used for identification of barbiturates in a number of pharmaceutical preparations.

DISCUSSION

The rate at which a solute travels through a gas chromatographic column depends on the vapor pressure of the pure compound and its activity coefficient in the stationary phase. With a nonpolar stationary phase where there is little or no interaction between solute and solvent, the separation in the column is largely a function of the vapor pressures of the pure substances. With an increase in the polarity of the stationary phase, various types of solute-solvent interaction take place, and the activity coefficient, expressing the lack of ideality of the solution, becomes an important component of the partition coefficient (36). For example, phenobarbital is eluted before cyclobarbital on an Apiezon column, as one might expect on the basis of their relative vapor pressures. On a polar column, such as a polyester or polyethylene glycol, the stationary phase causes more polarization of the phenyl ring than of the cyclohexenyl ring, and the elution sequence is reversed. A similar situation exists in the case of pentobarbital and vinbarbital which only differ by a double bond in one of the substituents in position 5.

Pentobarbital and secobarbital can easily be separated from their corresponding thio analogs on a nonpolar liquid (Fig. 1), but not on a polar liquid such as polyethylene glycol.

In Fig. 5 the relative retention times of a selection of barbiturates are plotted against the stationary phases arranged in the order of increasing polarity. The slopes of the curves indicate the degree of interaction between solute and solvent relative to that of pentobarbital. A positive slope would mean an increase in the activity coefficient over that of pentobarbital, and vice versa. As one would expect, the 1-substituted barbiturates, as well as the 2-thio barbiturates, exhibit negative slopes.

Dow Corning fluorosilicone fluid QF-1-0065 is a polar compound and has been used successfully for separation of steroids because of its great selectivity (37). It did not prove to be of any particular value for separation of barbiturates. It behaved as a very polar liquid in preventing the separation of pentobarbital and secobarbital from their corresponding thio analogs. On the other hand, it showed nonpolar behavior toward phenobarbital and cyclobarbital and similarly related compounds.

The gas chromatographic method gives promise of being an excellent tool, not only for analysis of barbiturates in pharmaceutical dosage forms, but also for fundamental studies of their rates and mechanisms of *in vitro* and *in vivo* transformations.

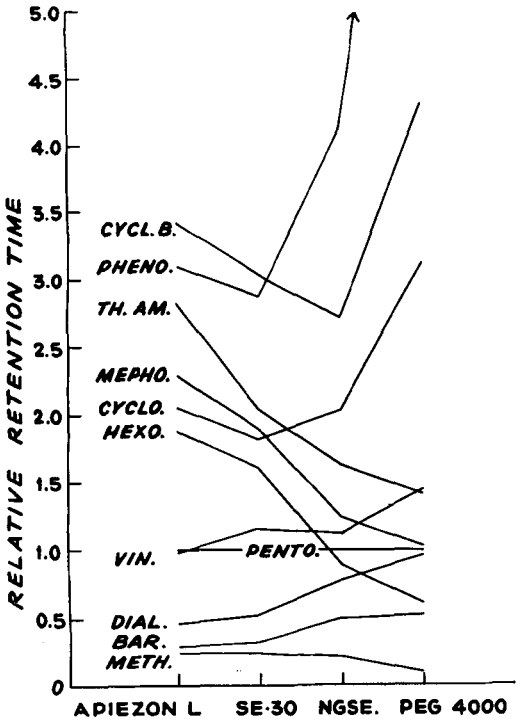

Fig. 5.—Relative retention times for 11 barbiturates plotted against stationary phases arranged in the order of increasing polarity. The experimental conditions and the names of the barbiturates refer to Table I.

SUMMARY

Twenty-three barbiturates and related compounds, including glutethimide and allylisopropylacetylurea have been gas chromatographed on columns containing stationary phases of varying polarity. By means of two columns, one with Apiezon L and one with a moderately polar polyester, most commonly used barbiturates can be separated. This method has been used for rapid identification of barbiturates in pharmaceutical preparations.

REFERENCES

(1) Castle, R. N., and Poe, C. F., *J. Am Chem. Soc.*, **66**, 1440(1944).
(2) McCutcheon, R. S., and Plein, E. M., THIS JOURNAL, **38**, 24(1949).
(3) DeWolff, C. J., *Acta Pharm. Intern.*, **2**, 107(1951).
(4) Heise, E., and Kimbel, K. H., *Arzneimittel-Forsch.*, **5**, 149(1955).
(5) Umberger, C. J., and Adams, G., *Anal. Chem.*, **24**, 1309(1952).
(6) Levi, L., and Hubley, C. E., *ibid.*, **28**, 1591(1956).
(7) Manning, J. J., and O'Brien, K. P., *Bull. Narcotics U. N. Dept. Social Affairs*, **10**, (No. 1) 25(1958).
(8) Cleverley, B., and Williams, P. P., *Chem. & Ind. London*, **1959**, 49.
(9) Cleverley, B., *Analyst*, **85**, 582(1960).
(10) Huang, T.-Y., *Dansk Tidsskr. Farm.*, Suppl. **1**, 1(1953).
(11) Hansen, E. J., and Jerslev, B., *ibid.*, **27**, 261(1953).
(12) Heiz, R., and Jerslev, B., *ibid.*, **28**, 11(1954).
(13) Hansen, E. J., and Jerslev, B., *ibid.*, **28**, 220(1954).
(14) Williams, P. P., *Anal. Chem.*, **31**, 140(1959).
(15) Penfrase, W. G., and Biles, J. A., THIS JOURNAL, **45**, 585(1956).
(16) Hultquist, M. E., and Poe, C. F., *Ind. Eng. Chem., Anal. Ed.*, **7**, 398(1935).
(17) Hultquist, M. E., Poe, C. F., and Witt, N. F., *ibid.*, **14**, 219(1942).

(18) Hultquist, M. E., Poe, C. F., and Witt, N. F., *J. Am. Chem. Soc.*, **67**, 688(1945).
(19) Keenan, G. L., *J. Assoc. Offic. Agr. Chemists*, **27**, 153(1944).
(20) Castle, R. N., THIS JOURNAL, **38**, 47(1949).
(21) Bush, M. T., *Microchem. J.*, **5**, 73(1961).
(22) Bräuniger, H., and Stens, B., *Pharmazie*, **12**, 794 (1957).
(23) Lach, J. L., Bhansali, K., and Blaug, S. M., THIS JOURNAL, **47**, 48(1958).
(24) Sahli, M., and Huber, M., *Sci. Pharm.*, **27**, 271(1959).
(25) Wickstrøm, A., and Salvesen, B., *J. Pharm. and Pharmacol.*, **4**, 98(1952).
(26) Allgén, L.-G., *Svensk Farm. Tidskr.*, **57**, 188(1953).
(27) Hübner, G., and Pfeil, E., *Z. physiol. Chem. Hoppe-Seyler's*, **296**, 225(1954).
(28) Relyveld, P., *Pharm. Weekblad*, **92**, 621(1957).
(29) Margasiński, Z., Ochab, S., Rafalowska, H., and Tyfczyńska, J., *Acta Polon. Pharm.*, **17**, 165(1960); through *Chem. Abstr.*, **54**, 20086(1960).
(30) Janák, J., *Collection Czechoslov. Chem. Commun.*, **25**, 1780(1960).
(31) Janák, J., *Nature*, **185**, 684(1960).
(32) Brochmann-Hanssen, E., and Svendsen, A. B., THIS JOURNAL, **50**, 804(1961).
(33) Bohemen, J., Langer, S. H., Perrett, R. H., and Purnell, J. H., *J. Chem. Soc.*, **494**, 2444(1961).
(34) Horning, E. C., Moscatelli, E. A., and Sweeley, C. C., *Chem. & Ind. London*, **1959**, 751.
(35) Hunter, I. R., Ng, H., and Pence, J. W., *Anal. Chem.*, **32**, 1757(1960).
(36) Keulemans, A. I. M., "Gas Chromatography," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1959.
(37) Vanden Heuvel, W. J. A., Haahti, E. O. A., and Horning, E. C., *J. Am. Chem. Soc.*, **83**, 1513(1961).

Color Stability of Tablet Formulations V

Effect of Ultraviolet Absorbers on the Photostability of Colored Tablets

By L. LACHMAN, T. URBANYI, S. WEINSTEIN, J. COOPER, and C. J. SWARTZ

The photostabilizing effect of the ultraviolet absorber 2,4-dihydroxybenzophenone for tablets colored with three certified dyes was evaluated. Two sources of light were employed; one approximating the spectral energy distribution of sunlight and the other simulating ordinary room illumination. The apparent rates of fading for FD&C Yellow No. 5, FD&C Blue No. 1, and FD&C Red No. 3 were determined under each light source. The relationship existing between the protective properties of the ultraviolet absorber, the absorption characteristics of the dyes, and the spectral energy distribution of the artificial light sources is discussed.

THE STATUS of certified colorants continues to be closely scrutinized by the Food and Drug Administration. With the delisting of FD&C Red No. 1, there remain 11 certified dyes available for use in foods, drugs, and cosmetics. Temporary authorization for the continued use of FD&C and D&C colorants has been given, subject to the possibility that such use may be terminated or restricted. In view of the pending restrictions inherent with the use of colorants, maximum utilization of the certified dyes has become essential. This has stimulated interest in the pharmaceutical industry for developing methods of improving the stability of the limited number of available certified dyes.

One approach for improving the photostability of dyestuffs has been the use of ultraviolet absorbing chemicals. Chemical compounds such as 3,5-dinitrobenzoic acid (1), benzoyl resorcinols (2), alkylated 2-hydroxyphenylbenzotriazole, and derivatives of 2-hydroxybenzophenones (3) have been employed effectively as photostabilizing agents in industries such as textiles, plastics, paints, and cosmetics. These compounds are selected because they possess high absorptivity and good stability in the 300–400 mμ ultraviolet region without absorbing in the 400–800 mμ visible region. Since many FD&C colorants absorb radiation in the near ultraviolet and visible region of the spectrum, the use of an ultraviolet absorber in conjunction with these colorants should result in some stabilizing influence. This effect, however, would be governed by the spectral energy distribution of the light source used and the absorption characteristics of the colorants.

This study was initiated to determine the influence of an ultraviolet absorber, namely 2,4-dihydroxybenzophenone, on the photostability of tablets colored with three FD&C dyes representing different chemical classes. The colorants were selected so that they would exhibit absorption maxima throughout the visible spectrum (430, 540, and 640 mμ). The photostability of

Received April 28, 1961, from the Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J.
Accepted for publication July 24, 1961.
Presented to the Scientific Section, A. PH. A., Chicago meeting, April 1961.