part, to the temperature of the injection port (ambient, relative to the oven temperature). As the injection port temperature was increased 20° or more over the oven temperature, the appearance of a peak on the trailing edge of the solvent front became pronounced. This peak was identified as 2-methyl-2-propyl-3-hydroxypropyl carbamate, a trace impurity in meprobamate and occasionally detected by TLC or GC methods. Holch and Gjaldbaek (11) confirmed that it also arises as a detectable degradation compound when the injection port is greater than 210°. Another commonly encountered compound is 2-methyl-2-propyl-1,3-propanediol. The "diol" is usually lost in the solvent peak and does not interfere in the analysis. Other known trace contaminants of meprobamate were tested and showed retention times that were either greater or lesser than meprobamate, thereby assuring no interference in the analytical procedure for the determination of meprobamate.

SUMMARY

A specific and rapid GLC method has been developed for the determination of meprobamate in bulk powder form and in tablets. The method for tablets utilizes a simple extraction procedure and subsequent combination with an internal standard. The use of tybamate as the internal standard permits the analysis of the related propanediol dicarbamates, carisoprodol and mebutamate, with no modifications necessary. Degradation of meprobamate in the GLC system is not apparent. Conditions are outlined for the operational parameters and extraction procedure.

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Determination of Isomeric Composition of Amphetamine Mixtures from Melting Points of Monohydrogen Succinate Salts

GERTRUDE J. LOWELL

Abstract □ A rapid and simple method for the identification of particular amphetamines and the determination of their isomeric composition is presented. The method involves the preparation of the monohydrogen succinate salt and uses the melting point of this derivative in the accurate determination of the isomeric composition of the amphetamine. Application of this procedure to actual samples as shown has affirmed the utility and validity of this method.

Keyphrases □ Amphetamine sulfate isomer mixtures—determination of isomeric composition from melting point of monohydrogen succinate salt □ Dextroamphetamine sulfate—determination in amphetamine sulfate mixtures from melting point of monohydrogen succinate salt □ Isomer mixtures, amphetamine sulfate—determination of components □ Succinic acid—amphetamine sulfate salt formation, determination of isomeric composition

The difference in the physiological response to *d*-amphetamine (dextroamphetamine) as compared to the *l*-stereoisomer has indicated the need for an isomer-measuring analytical procedure. Welsh (1, 2) discussed the problem from a phase diagram point of view and introduced a procedure, accurate to about 1%, involving the formation of the acetyl derivative and the determination of melting points. A recent paper (3) reviews the literature and describes a procedure for determining the optical isomer composition from the melting point of the benzoyl derivative.

The method reported here involves the preparation of the monohydrogen succinate salt of amphetamine and uses the melting point of this derivative to deter-

Table I—Melting-Point Behavior of Variable Composition Mixtures of *d*- and *l*-Amphetamine Monohydrogen Succinate Salts with Pure *d*- or *l*-Amphetamine Monohydrogen Succinate Salts

	Phase Diagram Point or Segment	Composition, % *d*	—Add Equal Amounts of Pure *d*— Resulting Composition, % *d*	Melting-Point Change	—Add Equal Amounts of Pure *l*— Resulting Composition, % *d*	Melting-Point Change
1	A	0	50	i[a]	0	—
2	AC	2	51	i	1	—
3	CD	4	52	i	2	—
4	DE	10	55	i	5	d
5	DE	30	65	i	15	d
6	E	50	75	d[b]	25	d
7	EF	70	85	d	35	i
8	EF	90	95	d	45	i
9	FG	96	98	—[c]	48	i
10	GJ	97	98.5	—	48.5	i
11	J	100	100	—	50	i

[a] i indicates increase. [b] d indicates decrease. [c] — indicates little or no change.

mine the isomeric composition of amphetamine. Application to actual samples has affirmed the utility and validity of this procedure. Advantages over other methods include speed and simplicity, since the derivative is readily formed and requires neither noxious reagents (*e.g.*, benzoyl chloride or acetyl chloride) nor special apparatus and instrumentation.

EXPERIMENTAL[1]

Chemicals and Reagents—The following were used: amphetamine sulfate[2], *d*, *l*, and *d,l;* ethyl ether, ACS reagent grade; succinic acid, ACS reagent grade; and a saturated solution of succinic acid in ether. (Shake about 0.5–1.0 g. of succinic acid with about 20 ml. of ether. To use, decant the supernate from the residual crystals. This solution contains about 6 mg. of succinic acid/ml.)

Procedures—If a dosage form is presented for analysis, the amphetamine may be isolated by aqueous extraction or another appropriate separation step.

With 1 *N* NaOH, make alkaline an aqueous solution containing about 25–50 mg. of amphetamine sulfate and extract with about 30 ml. of ether using a separator. (Smaller quantities such as 5.0 mg. may be taken if semimicro equipment is used.) Discard the aqueous portion. Wash the ether layer with water, discarding the wash water. Filter the ether through a cotton pledget wet with ether.

Add saturated succinic acid solution to the ether extract until no further precipitation is observed. Stir and allow the precipitate to flocculate. Filter the precipitate through paper and then wash it with about 10 ml. of ether. Let residual ether evaporate from the filter and then dry the precipitate on the filter at 105° for about 10 min.

The precipitate dries as a film and is easily separated from the filter paper. Reduce it to a powder and carefully determine the melting point within 0.3°. From a graph constructed by plotting the melting points of samples of different known isomeric compositions of amphetamine monohydrogen succinate (ordinate) *versus* the isomeric composition (abscissa) (Fig. 1), determine the unknown composition.

Since the observed melting point may correspond to at least two different compositions, a mixed melting point is necessary. If the initial melting point is above 150° (on line DEF), mix equal quantities of the unknown and *d*-amphetamine monohydrogen succinate salts and determine the melting point. If the initial melting point is below 150° (on line ACD or FGJ), carry out the mixed melting point using a mixture of equal quantities of the unknown and *l*-amphetamine monohydrogen succinate salts. Reference to Table I permits the direction of the difference in the melting points of the unknown and the mixture to be used for selection of the correct composition.

The nonaqueous titration of *d,l*-amphetamine monohydrogen succinate in glacial acetic acid with perchloric acid titrant was performed. The end-point potentials indicated that the succinate was being neutralized (4).

Where direct determination of optical rotation by polarimeter is desired, an aggregate sample may be extracted and the sulfate prepared as follows. Make a convenient size sample alkaline with sodium hydroxide and extract with ethyl ether. Wash with water and discard water layers. Shake the ether solution with not more than the stoichiometric equivalent of 0.1 *N* H_2SO_4. (It is safer to add a bit less than the theoretical amount since decomposition and discoloration may occur with an excess of sulfuric acid, which chars the material as it dries. The excess of amphetamine will volatilize.) Wash the ether layer once or twice with small amounts of water. Add the water washes to acid portions. Evaporate the combined aqueous layers to dryness in a small tared beaker. Dry 1–2 hr. in a 100° oven. Weigh and add water to make a 5% solution. The weight in milligrams divided by the factor 52.0 will give the volume of water to be added to make a 5% solution.

RESULTS AND DISCUSSION

Figure 1 presents the melting-point behavior of mixtures of *d*- and *l*-amphetamine monohydrogen succinates. The individual pure *d*- and *l*-salts both melt at 149–150°, whereas the racemic compound melts at 164.0°. Intermediate melting points correspond to mixtures between 50 and 100% composition. Two eutectic minima occur at about 96–97% relative purity (points C and G), and a maximum is noted at 50% *d,l*-composition (point E). Lines BCL and KGH are the eutectic temperature lines which are to be expected. These have not been experimentally determined and are not necessary for the analytical purpose of this paper but are included for completeness. As pointed out by Welsh (1), this is a relatively common type of phase diagram indicating that a racemic compound exists which forms a eutetic mixture with each of the optically active compounds.

This type of symmetrical diagram can introduce complication since one melting point may correspond to at least two or as many as four compositions. The latter possibility is clearly restricted to the eutectic regions where an isotherm may intersect four distinct composition lines. However, this situation is limited since the eutectics occur at high relative purity and involve melting points within a degree lower than either of the pure optically active compounds. Thus, the eutectics are important only when the melting point of the unknown falls within a degree of the pure compounds. Outside the eutectic regions, an observed melting point may indicate two possible compositions.

Since the initial melting point is not definite, the choice of the proper composition is made by means of a mixed melting point. Table I is a convenient summary of the behavior to be observed.

[1] Although any melting-point apparatus may be used, two specific units were used in this work. The Thomas-Hoover melting-point apparatus, No. 64 27-F10/6427-M10 (Arthur H. Thomas Co., Philadelphia, PA 19105), is a capillary tube setup which permits convenient simultaneous viewing of the sample in the tube and the thermometer. The Mettler FP1 instrument (Mettler Instrument Corp., Princeton, NJ 08540) requires the sample to be in a capillary tube and automatically displays the melting point digitally, which may be determined at any one of three rates of heating.

[2] Smith Kline and French Laboratories.

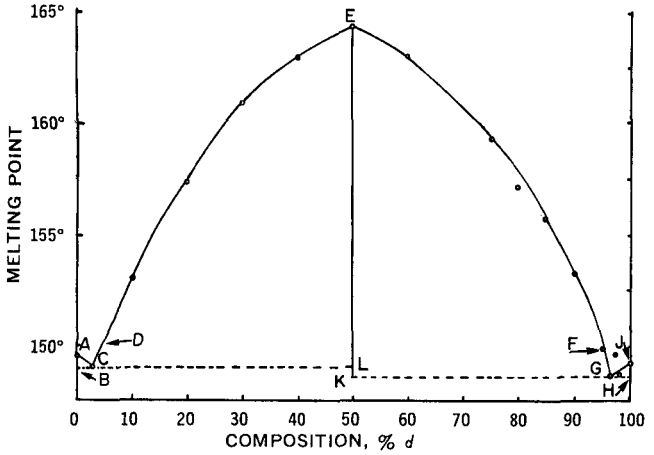

Figure 1—*Phase diagram describing the thermal behavior of the system* d-*amphetamine–*l-*amphetamine succinates.*

If the *d*-isomer–unknown mixture results in an increased melting point, then the composition is found at a point along line ACDE. A decreased melting point occurs if the composition is on line EF. No observable change may result if the unknown has a composition along line FGJ unless melting points are very carefully taken. This latter observation may be further resolved by a second mixed melting point using *l*-amphetamine monohydrogen succinate. In this case, a mixture with a composition on FGJ will show an increased melting point.

Analysis of the *d,l*-racemic compound was found to have the base and acid present in a 1:1 stoichiometric ratio. Nonaqueous titration of the racemic salt in glacial acetic acid, which involves the neutralization of the basic monohydrogen succinate anion by perchloric acid, verified this ratio as did gravimetric observations. This establishes the salts as *d*-, *l*-, or *d,l*-amphetamine monohydrogen succinate.

The analytical utility of the procedure depends, in part, on the attainable sensitivity. In this case, a 1% change in composition produces a 0.3° change in melting point. Therefore, since the melting point method used in this work showed a reproducibility of 0.5°, a change in composition of 1% would be detected.

The method of melting-point determination depends upon the preference of the laboratory. Any procedure used carefully can be satisfactory. In this laboratory, the Mettler automatic digital capillary apparatus gave consistent results with less than 1° variation among replicate values. This instrumentation does not require prolonged attention. Samples in which the isomeric composition is of interest are usually large enough to fill a capillary tube to the required height of about 3 mm. The Thomas-Hoover apparatus was suitable but gave melting points about 1° lower than the automatic device. If only a small quantity of derivative is available, *e.g.*, the analysis of a single tablet or less, a Kofler hot stage, properly calibrated, could be used.

This procedure was recently tested when a sample of *l*-amphetamine sulfate tablets was found to contain some racemate when examined microscopically with the aid of gold chloride reagents (5). As described for this procedure, the succinate was formed, isolated, and melted at 156.2°, which indicates a composition of 82.5% *l*-isomer[3]. Extraction of an aggregate sample followed by optical rotation measurement indicated a composition of 83.4% *l*-isomer (based on a specific rotation of −23.5°). This is excellent agreement considering the separation step used before the rotation measurement and the lack of sensitivity of the latter method.

In addition to the utility of this succinate procedure in determining the *l*- and *d*-compositions of amphetamine, this method is useful in differentiating amphetamine from methamphetamine. The latter does not form an insoluble succinate in ether, even though initial transient opalescence may result. To confirm the negative observation, oxalic acid (in ether) may then be added which does result in the precipitation of the oxalate of methamphetamine.

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[3] See *Procedures* section.