In view of the foregoing evidence, the aromatization of 9α-hydroxy and 19-hydroxyandrost-4-en-3,17-dione involve a direct 1,2-dehydrogenation to give vinylogs of β-hydroxyketones which undergo spontaneous nonenzymic rearrangements (reverse aldolization) to give their respective phenols. The microbial aromatizing system differs from that of the human placental microsomes in that (*a*) the relative rate of oxidation follows the order 19-nor>19-oxo>19-hydroxy, (*b*) oxygen and $NADPH_2$ are not required in the aromatization reaction, and (*c*) a suitable electron acceptor characteristic of flavoproteins is needed.

REFERENCES

(1) Dodson, R. M., and Muir, R. D., *J. Am. Chem. Soc.* **83**, 4627(1961).
(2) Ryan, K. J., *J. Biol. Chem.*, **234**, 268(1959).
(3) Sih, C. J., *Biochem. Biophys. Res. Commun.*, **7**, 87(1962).
(4) Sih, C. J., *J. Org. Chem.*, **26**, 4716(1961).
(5) Fried, J., and Sabo, E. F., *J. Am. Chem. Soc.*, **79**, 1130(1957).
(6) Gornall, A. G., Bardawill, C. J., and David, H. M. *J. Biol. Chem.*, **177**, 751(1949).
(7) Stowe, B. B., and Thimann, K. V., *Arch. Biochem. Biophys.*, **51**, 499(1954).
(8) Wood, W. A., and Gilford, S. R., *Anal. Biochem.*, **2**, 589(1961).
(9) Sih, C. J., and Bennett, R. E., *Biochim. Biophys. Acta*, **56**, 584(1962).
(10) Zaffaroni, A., Burton, R. B., and Keutman, E. H., *Science*, **111**, 6(1950).
(11) Sih, C. J., and Laval, J., *Biochim. Biophys. Acta*, **64**, 409(1962).
(12) Loke, K. H., Marrian, G. J., and Watson, E. J. D., *Biochem. J.*, **71**, 43(1955).
(13) Sih, C. J., *Biochim. Biophys. Acta*, **62**, 541(1962).
(14) Volpp, G., and Tamm, Ch., *Helv. Chim. Acta*, **42**, 1408(1959).
(15) Beinert, H., and Crane, F., "Inorganic Nitrogen Metabolism," The Johns Hopkins Press, Baltimore, Md., 1956, p. 601.
(16) Ringold, H. J., and Turner, A., *Chem. Ind.*, 1962 (No. 5), 211.
(17) Ehrenstein, M., and Otto, K., *J. Org. Chem.*, **24**, 2006(1959).
(18) Longchampt, J. E., Hayano, M., Ehrenstein, M., and Dorfman, R. I., *Endocrinology*, **67**, 843(1960).

Identification of Sympathomimetic Amines as Tetraphenylborates

By J. E. SINSHEIMER and EDWARD SMITH†

The tetraphenylborate salts of sympathomimetic amines are readily isolated in a highly pure state even from low concentrations in complex mixtures. Therefore, melting points, infrared, and ultraviolet spectral characteristics of these salts were studied as an aid to the identification of the medicinally important sympathomimetic amines.

CHATTEN AND LEVI (1) and Fischer and Plein (2) have prepared derivatives for the identification of sympathomimetic amines and have listed references for the identification of these amines. Characterization through tetraphenylborate (TPB) salts would be a valuable addition to these present methods for identifying sympathomimetic amines.

Isolation of organic bases as their TPB salts and subsequent identification by melting point is well established (3–12). Determination of various alkaloids and chemotherapeutic agents by ultraviolet spectra of their TPB salts has also been reported (13, 14). Chatten, Pernarowski, and Levi (15) have prepared the TPB salts of a series of local anesthetics and have reported their ultraviolet and infrared spectra.

In general, TPB derivatives of organic bases

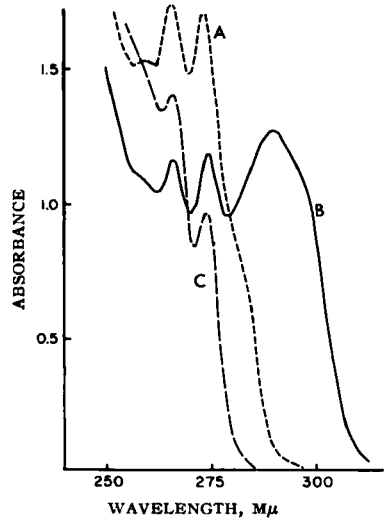

Fig. 1.—Ultraviolet spectra in methanol. Key: A, phenylephrine TPB ($2.53 \times 10^{-4}M$); B, methoxamine TPB ($6.46 \times 10^{-4}M$); C, ephedrine TPB ($4.83 \times 10^{-4}M$).

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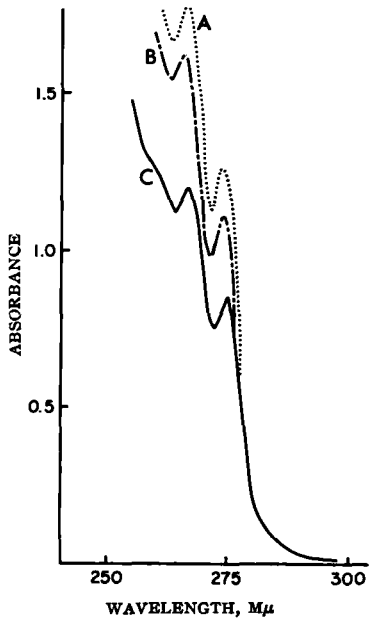

Fig. 2.—Ultraviolet spectra in methanol. Key: A, tetrahydrozoline TPB ($5.66 \times 10^{-4}M$); B, amphetamine TPB ($5.72 \times 10^{-4}M$): C, propylhexedrine TPB ($4.21 \times 10^{-4}M$).

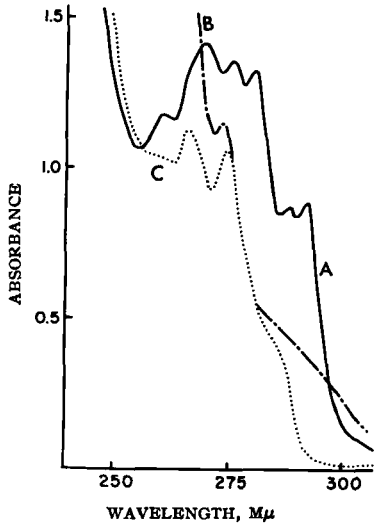

Fig. 3.—Ultraviolet spectra in methanol. Key: A, naphazoline TPB ($3.52 \times 10^{-4}M$); B, diethylpropion TPB ($5.07 \times 10^{-4}M$); C, hydroxyamphetamine TPB ($3.78 \times 10^{-4}M$).

are readily isolated in a pure form even from low concentrations in complex mixtures. Therefore, the melting points and spectral properties of these salts are recorded in this paper as an aid to the identification of this important group of amines.

EXPERIMENTAL

Apparatus and Materials.—Melting points were determined by the U.S.P. capillary tube method with a modified Thiele tube and a calibrated ASTM thermometer. Ultraviolet spectra were determined with the use of a Cary model 11 recording quartz spectrophotometer. Infrared spectral studies were made on Perkin-Elmer model 21 infrared spectrophotometers with sodium chloride or calcium fluoride optics.

Amine TPB salts were prepared as previously described (16) by addition of sodium TPB solution to acetate buffered solutions of the amine. The sympathomimetic amine salts studied and their melting points are listed in Table I. Reagents were of A.R. or U.S.P. grade and were used without additional purification.

The pH 4.6 acetate buffer used for preparation of

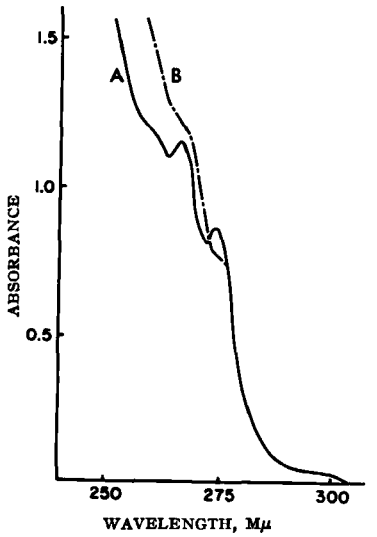

Fig. 4.—Effect of solvent change on sodium TPB spectra ($4 \times 10^{-4}M$). Key: A, methanol; B, acetate buffer.

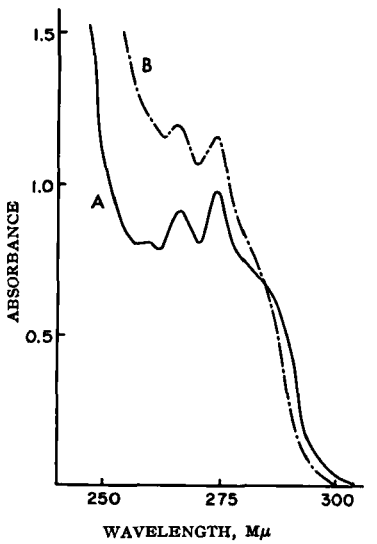

Fig. 5.—Effect of solvent change on epinephrine TPB spectra. Key: A, methanol ($2.35 \times 10^{-4}M$); B, acetate buffer ($2.87 \times 10^{-4}M$).

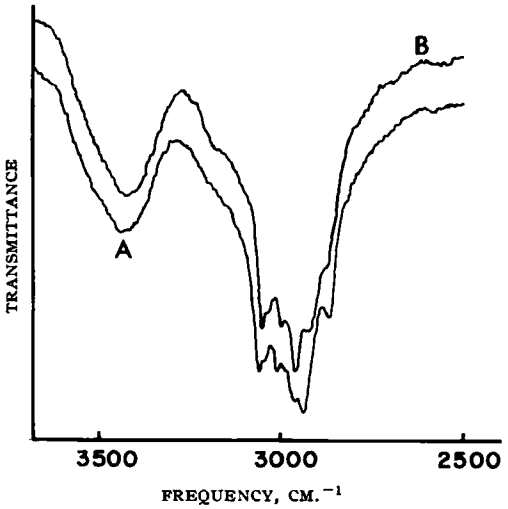

Fig. 6.—Infrared spectra with CaF_2 optics. Key: A, tuaminoheptane TPB; B, methylhexaneamine TPB.

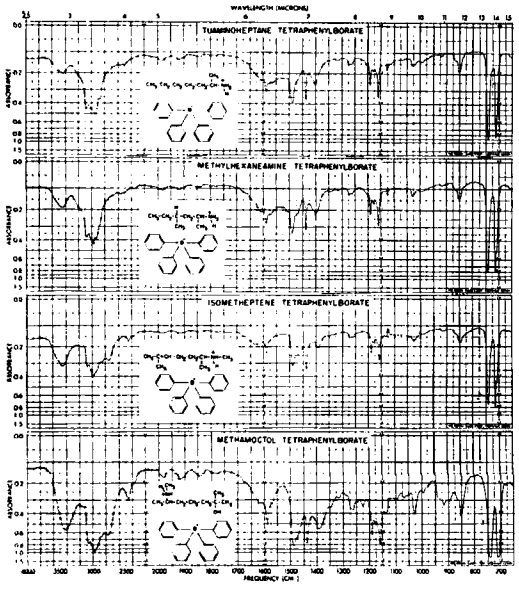

Fig. 7.—Infrared spectra.

the TPB salts and for the study of ultraviolet absorption spectra contained 0.8 mole each of acetic acid and sodium acetate per liter.

Determination of Spectra.—Ultraviolet absorption spectra were determined from 400 to 220 mμ in anhydrous methanol and in an acetate buffer. The infrared spectra were obtained from 4000 to

Table I.—Melting Points of Sympathomimetic Amine Salts

Compd.		M.p., °C. Tetraphenylborate		
		Compd.	Literature	Found
Amphetamine[a]	H_2SO_4	>300 dec.	114–116(10, 11)	114–116
Cyclopentamine[b]	HCl	113–115	...	149–151
Diethylpropion[c]	HCl	168–170	...	128–130 dec.
Ephedrine[n]	H_2SO_4	243–245 dec.	124(12) 135–138(5) 135 to 136.5(10)	128 to 129.5
Epinephrine[p]	...	211–212	...	135–138 dec.
Hydroxyamphetamine[a]	HBr	189–192	...	98–101
Isometheptene	Mucate	150–152	...	119.5 to 121
Isoproterenol[e]	H_2SO_4	127–129	...	119–120
Mephentermine[f]	H_2SO_4	215–220	...	158–159 dec.
Metaraminol[g]	Bitartrate	176–177	...	107–109
Methamoctol[d]	Mucate	157–158	...	106–108
Methamphetamine[a]	HCl	171–175	...	127–128
Methoxamine[h]	HCl	212–216	...	106–108
Methoxyphenamine[i]	HCl	124–128	...	132–134
Methylhexaneamine[b]	HCl	132–134	...	115–117
Naphazoline[j]	HCl	256–260	...	198–200
Nylidrin[k]	HCl	225–228 dec.	no precipitate	
Phenmetrazine[l]	HCl	176–178	...	149 to 150.5 dec.
Phenylephrine[q]	HCl	139–143	143 to 144.5(10)	141.5 to 142.5
Phenylethanolamine[r]	HCl	211–212	...	95 to 96.5
Phenylethylamine[o]	HCl	215–217	173–175(8)	173–175
Phenylpropanolamine[o]	HCl	195–196	...	119–120
β-Phenyl-*n*-propylamine[c]	HCl	123–124	...	157–159
Phenylpropylmethylamine[c]	HCl	144–148	...	128–129
Propylhexedrine[a]	HCl	127–128	...	143–145
Synephrine[s]	Tartrate	188–192	133.5 to 134(10)	133.4 to 134
Tetrahydrozoline[m]	HCl	255–257	...	200–202
Tuaminoheptane[b]	H_2SO_4	131–133	...	112–114
Tyramine[o]	HCl	266–269	129(4)	123 to 124.5

[a] Smith Kline and French Laboratories, Philadelphia, Pa. [b] Eli Lilly and Co., Indianapolis, Ind. [c] The Wm. S. Merrell Co., Cincinnati, Ohio. [d] Knoll Pharmaceutical Co., Orange, N. J. [e] Abbott Laboratories, North Chicago, Ill. [f] Wyeth Laboratories, Philadelphia, Pa. [g] Merck Sharp and Dohme Laboratories, West Point, Pa. [h] Burroughs Wellcome Co., Tuckahoe, N. Y. [i] The Upjohn Co., Kalamazoo, Mich. [j] Ciba Pharmaceutical Products, Inc., Summit, N. J. [k] U. S. Vitamin and Pharmaceutical Corp., New York, N. Y. [l] Geigy Pharmaceuticals, Yonkers, N. Y. [m] Chas. Pfizer and Co., Inc., Brooklyn, N. Y. Grateful acknowledgement is made for the compounds supplied by these manufacturers. The balance of the compounds were purchased as follows: [n] Merck and Co., Inc., Rahway, N. J. [o] Eastman Kodak, Rochester, N. Y. [p] Matheson, Coleman and Bell, East Rutherford, N. J. [q] Winthrop Laboratories, New York, N. Y. [r] K & K Laboratories, Jamaica, N. Y. [s] L. Light and Co., Colnbrook, England.

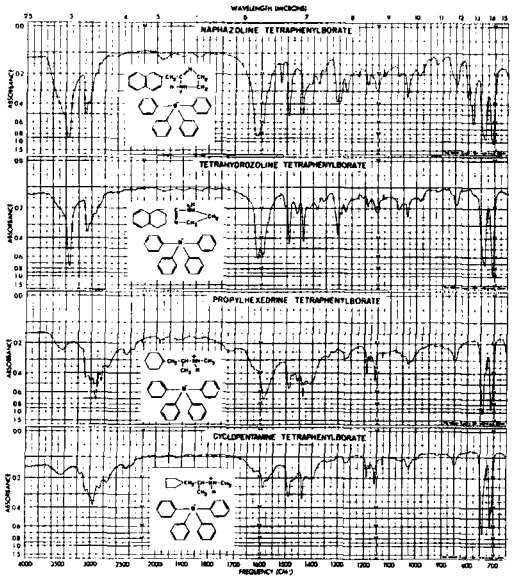

Fig. 8.—Infrared spectra.

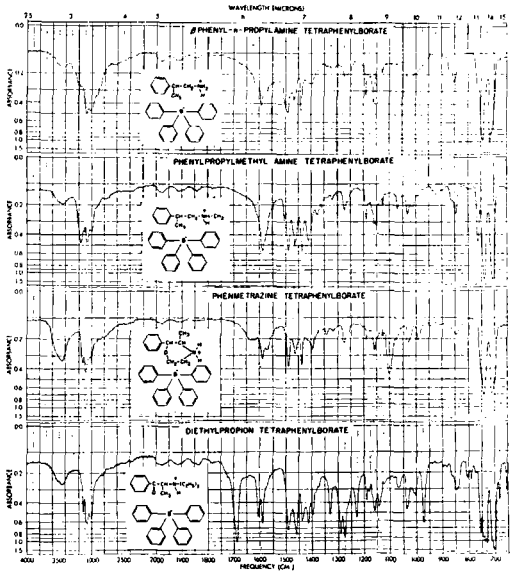

Fig. 10.—Infrared spectra.

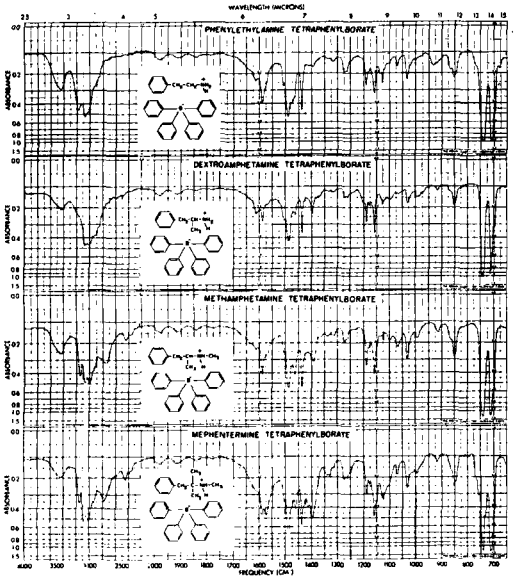

Fig. 9.—Infrared spectra.

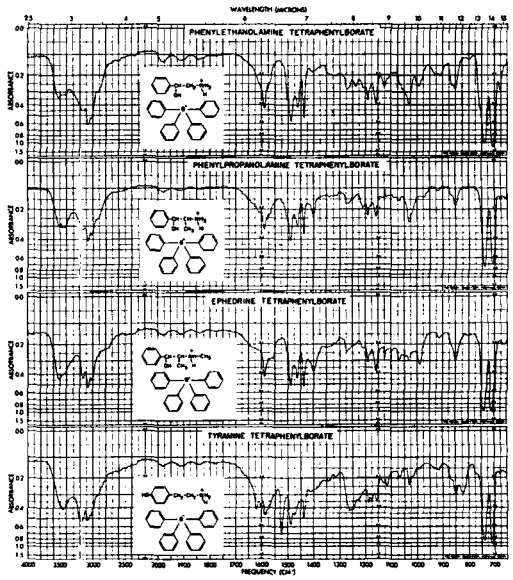

Fig. 11.—Infrared spectra.

650 cm.$^{-1}$ with the use of KBr disks. Approximately 1-mg. samples of dried TPB salts were mixed with 200 mg. of potassium bromide for 30 seconds with a steel capsule and pestle on a Wig-L-Bug.[1] The mixed sample in a KBr die was subjected to vacuum for 5 minutes before and also while being pressed at 20,000 p.s.i. for 5 minutes.

DISCUSSION

The rate of formation of the TPB salts was the same as in the amperometric titration of these amines with TPB (16). Precipitates formed rapidly, except for phenol and catechol derivatives. Phenol derivatives generally precipitated after 1 hour, except with metaraminol and nylidrin. No TPB salt could be obtained from nylidrin, while TPB salts of metaraminol and catechol derivatives could only be prepared after stirring for several hours.

Washing TPB salts free of excess reagent and drying under vacuum yields compounds which are sufficiently pure for identification purposes (8, 9, 15). Crane has found crystallization to be unnecessary and to decompose TPB salts in some instances (8).

The melting or decomposition points observed are listed in Table I.

[1] Crescent Dental Manufacturing Co.

Ultraviolet Spectra. —The ultraviolet spectra of amine TPB salts are summations of the absorbance of TPB and parent amine. Therefore, any strong absorbance maxima of the amine can still be detected; characterization of the U.V. spectra of sympathomimetic amine TPB salts follows that of the spectra of the parent amine, *per se*, as reported for example by Thies and Özbilici (17). Phenylalkyl, phenolic, and catechol amine derivatives can be distinguished by changes in absorbance in the region of the TPB maxima (266 and 274 mμ). Typical spectra of TPB salts are given in Figs. 1-3. Figure 4 illustrates the contribution due to TPB. Table II is a summary of molar absorptivities of TPB salts of the amines and includes sodium TPB for comparison.

The ultraviolet characteristics of most TPB salts in acetate buffer are similar to those in methanol. The disappearance of the minima of the TPB absorbance (264 and 272 mμ) is the predominant change and results in the appearance of shoulders instead of peaks as illustrated in Fig. 4. Phenol derivatives as illustrated in Fig. 5 represent an exception in that minima were observed and the relative intensity of the peaks was altered by a change to the acidic media.

Infrared Measurements.—The TPB ion exhibits absorbance characteristics due to monosubstituted phenyl and boron-aryl (18, 19) groups as shown by Chatten, Pernarowski, and Levi (15) for sodium TPB. It is noted that potassium bromide itself readily absorbs moisture, producing additional absorbance in the 3500 and 1650 cm.$^{-1}$ regions. Although there is a similarity in spectra because of the absorbance due to the TPB group, no two spectra are identical.

The differentiation of closely related compounds can be illustrated by a comparison of the spectra of tuaminoheptane and methylhexaneamine tetraphenylborates. As shown in Fig. 6, calcium fluoride optics accentuate spectral differences for the two compounds in the CH stretching region, a difference which is difficult to detect with sodium chloride optics as illustrated by the spectra of these two salts in Fig. 7. Methylhexaneamine has a definite increase in the CH_3 stretching absorbance at 2962

TABLE II.—ULTRAVIOLET MOLAR ABSORPTIVITIES OF TETRAPHENYLBORATES IN METHANOL

Compd.	Molar Absorptivity at 266 mμ[a]	Molar Absorptivity at 274 mμ[a]
PHENYLALKYLAMINES		
Amphetamine	2710	1860
Diethylpropion	...	2260
Mephentermine	2800	1940
Methamphetamine	2960	2020
Phenmetrazine	2900	1970
Phenylethylamine	2710	1850
β-Phenyl-*n*-propylamine	2690	1850
Phenylpropylmethylamine	2810	1930
BENZYLIC HYDROXYL AMINES		
Ephedrine	2920	2010
Phenylethanolamine	2770	1910
Phenylpropanolamine	2820	1940
PHENOLIC AMINES		
Hydroxyamphetamine	2970	2800
Metaraminol	3820	3670
Phenylephrine	3440	3360
Synephrine	3380	3080
Tyramine	3590	3400
CATECHOLAMINES		
Epinephrine	3910	4180 (274.5 mμ)
Isoproterenol	3910	4250 (275 mμ)
METHOXYPHENYLALKYLAMINES		
Methoxamine	2730	2770 (274.5 mμ) 2790 (291 mμ)
Methoxphenamine	2600	2400 (273.5 mμ)
ALIPHATIC AMINES		
Cyclopentamine	2790	1960
Isometheptene	2850	2000
Methamoctol	2710	1900
Methylhexaneamine	2780	1970
Propylhexedrine	2860	2040
Tuaminoheptane	2690	1890
IMIDAZOLES		
Naphazoline	2490 (291 mμ) 3740 (280 mμ) 4000 (268 mμ)	156 (312 mμ) 2460 (288 mμ) 3830 (275.5 mμ) 3324 (261 mμ)
Tetrahydrozoline	3150	2250
SODIUM	2980	2070

[a] Exception to the 266 mμ and 274 mμ maxima are given in parenthesis; absorptivities then correspond to these different maxima.

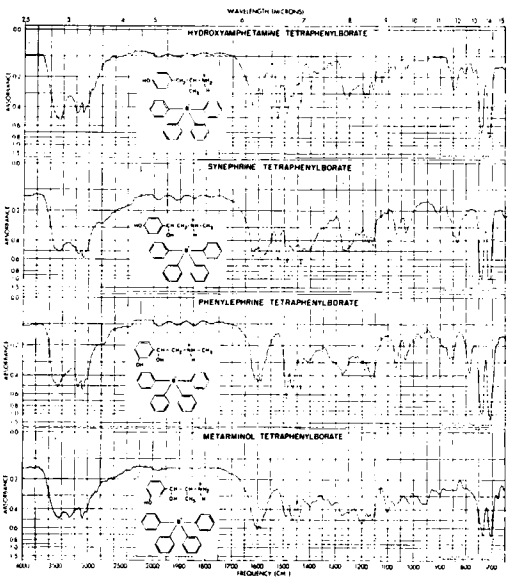

Fig. 12.—Infrared spectra.

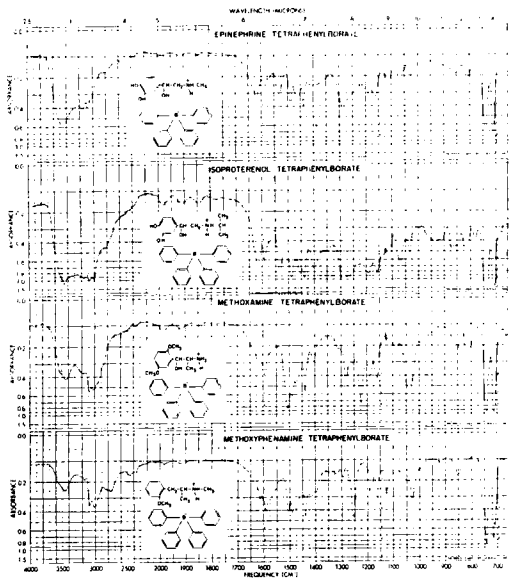

Fig. 13.—Infrared spectra.

and 2872 cm.$^{-1}$ compared to the CH_2 stretching absorbance at 2926 and 2853 cm.$^{-1}$ This is consistent with the increased CH_3 to CH_2 ratio from 2:4 in tuaminoheptane to 3:2 in methylhexaneamine.

The spectra of sympathomimetic amine TPB salts obtained with potassium bromide pellets on the model 21 spectrophotometer with sodium chloride optics are illustrated in Figs. 7–13. It can be seen from these spectra that TPB salts serve as a convenient medium for the isolation and identification of various members of a series of amines.

SUMMARY

The preparation of TPB salts of sympathomimetic amines together with their melting points, ultraviolet, and infrared spectra have been shown to be useful for the identification of these amines.

REFERENCES

(1) Chatten, L. G., and Levi, L., *Anal. Chem.*, **31**, 1581(1959).
(2) Fischer, W. H., and Plein, E. M., THIS JOURNAL, **44**, 313(1955).
(3) Flaschka, H., Barnard, A. J. Jr., "Tetraphenylboron as An Analytical Reagent," in "Advances in Analytical Chemistry and Instrumentation," Vol. I, Reilley, C. N., ed., Interscience Publishers, Inc., New York, 1960, pp. 1–117.
(4) Barnard, A. J., Jr., and Wendlant, W. W., *Revista Soc. Quim. Mex.*, **3**, 269(1959).
(5) Scott, W. E., Doukas, H. M., and Schaffer, P. S., THIS JOURNAL, **45**, 568(1956).
(6) Koehler, H. M., and Feldmann, E. G., *Anal. Chem.*, **32**, 28(1960).
(7) Fischer, R., and Karawia, M. S., *Mikrochim. Acta*, **1953**, 366.
(8) Crane, F. E., *Anal. Chem.*, **28**, 1794(1956).
(9) *Ibid.*, **30**, 1426(1958).
(10) Espersen, T., *Dansk. Tidsskr. Farm.*, **32**, 99(1958).
(11) Brauniger, H., and Spangenberg, K., *Pharmazie*, **9**, 623(1954).
(12) Keller, W., and Weiss, F., *ibid.*, **12**, 19(1957).
(13) Gautier, J. A., Renault, J., and Rabiant, J., *Ann. Pharm. Franc.*, **17**, 401(1959).
(14) *Ibid.*, **17**, 491(1959).
(15) Chatten, L. G., Pernarowski, M., and Levi, L., THIS JOURNAL, **48**, 276(1959).
(16) Smith, E., Worrell, L. F., and Sinsheimer, J. E., *Anal. Chem.*, **35**, 58(1963).
(17) Thies, H., and Özbilici, Z., *Arch. Pharm.*, **295**, 194(1962).
(18) Bellamy, L. J., "The Infra-red Spectra of Complex Molecules," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1958.
(19) Cross, A. D., "Introduction to Practical Infra-red Spectroscopy," Butterworths Scientific Publications, London, 1960, p. 74.