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Determination of the Optical Isomers of Ethambutol (Myambutol) and 2-Amino-1-butanol by Gas-liquid Chromatography

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Abstract □Our need for a convenient method of analytical estimation of the precise optical purity of *d*-2-aminobutanol (*d*-2AB) and d-ethambutol has prompted us to examine in detail the preparation and G.L.C. separation of the N-TFA-L-prolyl derivatives of their optical isomers (*d*-and *l*-2AB, *d*-, meso-and *l*-ethambutol). Silicon OV-1 columns were used for the G.L.C. separation.

Keywords □ G.L.C. separation of the optical isomers of 2-amino-1-butanol and ethambutol.

Antituberculostatic chemotherapeutic agent, Ethambutol (*d*-2,2'-ethylenediiminodi-1-butanol) was first found out by Wilkinson *et al.* in 1961,[1] to have an excellent antituberculostatic activity. Currently, *d*-form of ethambutol has been widely used clinically as antituberculostatic agent but *l*-form or meso-form of this compound possesses little potency and of no practical use for their side effects.[2]

Ethambutol (ETB) is prepared by brief heating of ethylene dichloride with excess *d*-2-amino-1-butanol (*d*-2AB).[1] *d*-2AB is obtained by resolution of racemic 2AB-which is prepared from 3,4-epoxy-1-butene-with glutamic acid or tartaric acid.[2~3] But owing to levo impurity in the *d*-2AB, the levo and meso isomers also are appeared.[1]

The practical importance of the preparation and the quantitative analysis (*i.e.* optical purity) of the *d*-form of ethambutol, free of levo and meso forms, needs no dilation. Accordingly, the determination of *d*-2AB's optical purity is very important, too. So Gas-Liquid-Chromatographic (G.L.C.) separation of the optical isomers of 2-AB (*d*-, *l*-) and ETB (*d*-, meso-, *l*-) has been investigated.

The separation of optically active amines by G. L. C. can be achieved by using either an optically active stationary phase, after making derivatives with a suitable optically inactive reagent,[4~6] or an optically active reagent to form diastereoisomers followed by chromatography on an optically inactive stationary phase.[7~17]

N-Trifluoroacetyl-L-prolyl chloride (TPC) is such an optically active reagent. It has been used for the resolution of numerous asymmetric amines, but the purpose of those studies was mainly to correlate the stereochemical features of the asymmetric compounds to the relative retention times of their diastereoisomeric derivatives.

We now report investigations designed to provide a method for the separation of

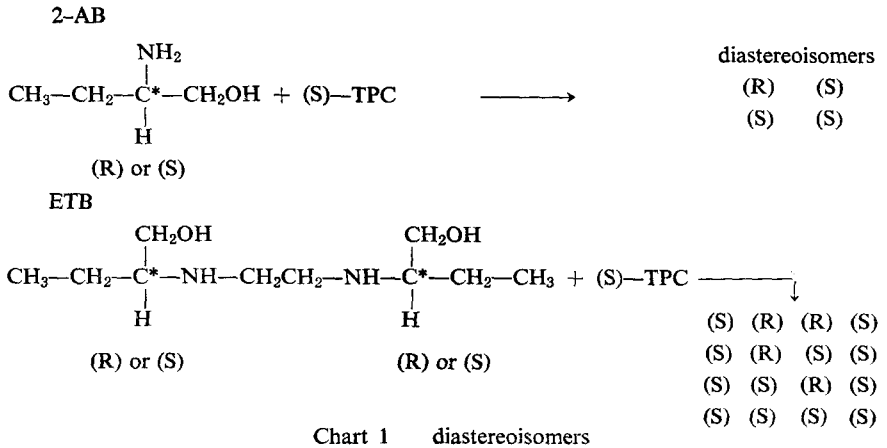


Chart 1 diastereoisomers

the optical isomers of 2-AB and ETB by means of their N-Trifluoroacetyl-L-prolyl (TP) derivatives and by using silicon OV-1 column.

EXPERIMENTAL

Apparatus

2-AB

G.L.C

Equipment : Pye Unicam GCV Chromatography

Column : 2.7m long × 4mm i.d. glass with 3% OV-1 on 100-200 mesh Diatomile CQ. 155°C isothermal.

Injector : 160°C

Detector : FID 200°C

Carrier gas : N_2 25ml/min

G.C Mass

Equipment : Aei MS 1073 & Pye Unicam 104 G.C.

Column : 2m long × 4mm i.d. 3% OV-1 on 80-100 mesh 160°C isothermal.

Carrier gas : He 27ml/min

Separator : 200°C

Electron energy : 70 eV

ETB

G.L.C

Equipment : Pye Unicam GCV Chromatography

Column : 2.1m long × 4mm i. d. glass with 1.5% OV-1 on 80-100 mesh, shimalite W(AW-DMCS) (201D), 240°C isothermal.

Injector : 240°C

Detector : FID 280°C

Carrier gas : N_2 25ml/min

G.C Mass

Equipment : Shimadzu LKB-9000

Column : 3m long × 4mm i. d. 1% OV-1 on 80-100 mesh Gaschrome Q 240°C isothermal.

Carrier gas : He 25ml/min

Separator : 280°C

Electron energy : 70eV

Materials

The optical isomers of ethambutol

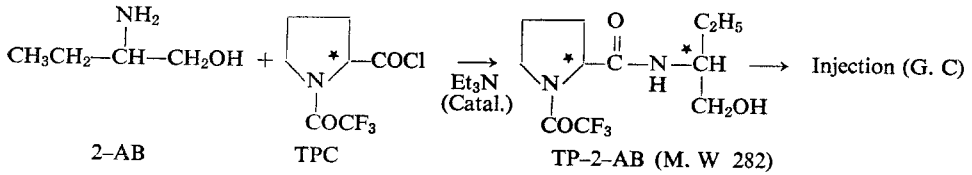


Chart 2; The preparation of TP derivatives of 2-AB

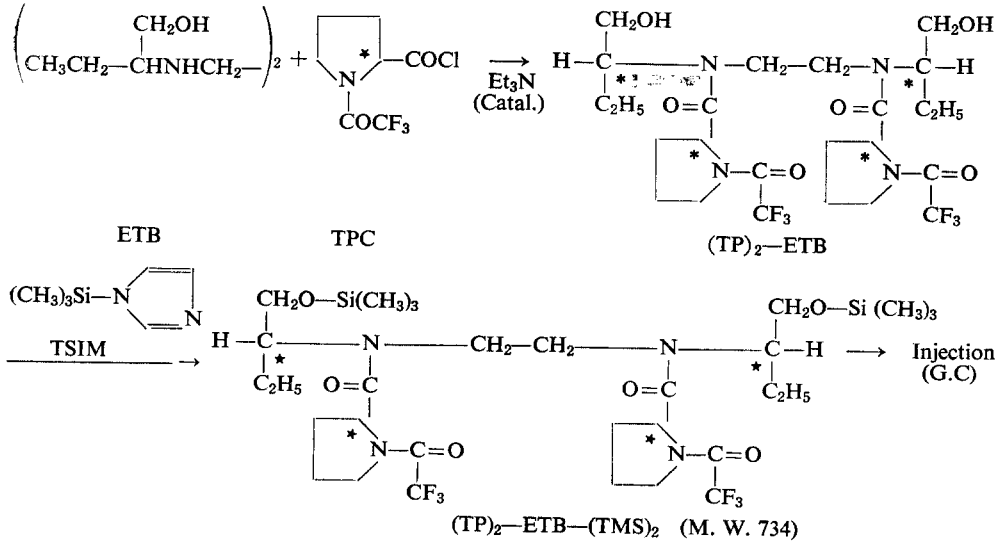


Chart 3; The preparation of $(TP)_2$—ETB—$(TMS)_2$.

dihydrochloride and 2-amino-1-butanol were kindly provided from Chong Kun Dang Cooperation and Yu-Han Cooperation. Trifluoroacetyl anhydride (TFAA) and N-Trimethylsilylimidazole (TSIM) were obtained from Pierce Chemical Cooperation and L-proline from Ajinomoto Cooperation, Tokyo. All other reagents used in this study were analytical grade.

Synthesis of N-Trifluoroacetyl-L-prolyl chloride

This compound was prepared following a modification of J. C. Dabrowiak[7] and William A. Bonner.[9]

The chloroformic 0.1M solution of L-(S)-TPC was stored in a freezer (-20°C) to its use.

Preparation of TP Derivatives of 2-AB

One hundred microliters of 2-AB was quantitatively taken up in 50ml of chloroform. 1.5ml of the solution (*i.e.* 3μl of 2-AB) was placed in a 50mm culture tube, and the solvent was removed under a stream of dry nitrogen gas. 0.7ml of TPC and 50μl of triethylamine (Et_3N) were added to the residual oil which remained. The sealed tube was allowed to stand at room temperature. After 3min, a 1 to 3μl portion of this solution was injected into the gas chromatography, and the response analyzed as discussed in the text.

Preparation of $(TP)_2$-ETB-$(TMS)_2$

Two hundred milligrams of ethambutol dihydrochloride were transfered to a 125ml

separator with the aid of 10ml of 2N-NaOH solution. The contents were swirled to form a fine suspension, and extracted with five 25ml portions of chloroform. After the chloroform extracts were filtered through anhydrous sodium sulfate, the total volume was arranged to be 100ml. 2.5ml of the solution (*i.e.* 5mg of ETB. 2HCl, 3.7mg of ETB free base) was placed in a 50mm culture tube, and the solvent was removed under a stream of dry nitrogen gas. 0.8–1.0ml of TPC and 0.1ml of triethylamine were added to the residual solid which remained. The sealed tube was allowed to stand at room temperature. After 15min, 200μl of TSIM was added, and allowed to stand for 5min at room temperature. A 2μl portion of this solution was injected into gas chromatography and the response analyzed as discussed in the text.

RESULTS AND DISCUSSION

2-*AB*

Evaluation of the optimum condition for the formation of TP-2AB with Et_3N and L-TPC was made by comparing the peak area of TP-d-2AB relative to that of n-$C_{18}H_{38}$ used as internal standard. The results are given in Tables I and II.

Table I: Effect of the amount of the L-TPC on peak area ratio (TP-2AB).

L-TPC added	d-2AB/$C_{18}H_{38}$ (int. st.)
0.3ml	1.388
0.5ml	1.466
0.75ml	1.466
1.0ml	1.466

*d-2AB 3μl, TEA 0.05ml, reaction time 3min.

Table II: Effect of the amount of the Et_3N on peak area ratio (TP-2AB)

Et_3N added	d-2AB/$C_{18}H_{38}$ (int. st.)
0.05ml	1.466
0.10ml	1.466
0.15ml	1.466

* d-2AB 3μl, TPC 0.7 ml, reaction time 3min.

As seen in Tables I and II when 0.5–0.75 ml of L-TPC and 0.05 ml of triethylamine were added to 3μl of 2-AB, the suitable peaks were obtained. And the reaction immediately completes, so as to be within 3 min.

Though the amount of Et_3N was increased in proportion to that of L-TPC, the peak area ratio didn't increase any more.

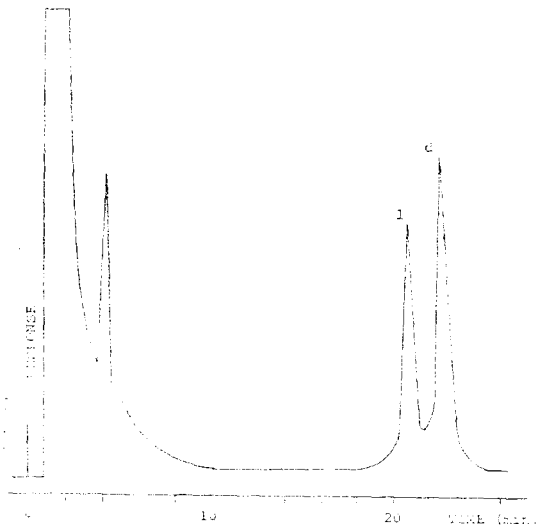


Fig. 1: Gas chromatogram of TP-2-AB diastereoisomers.
Instrument: Pye Unicam GCV chro.
Column: 2.7cm×4mm i. d., glass with 3% OV-1 on 100–120 mesh diatomile CQ, 155°C isothermal
Injector: 160°C, detector: FID 200°C
Carrier gas: N_2 25ml/min, sample size:1μl.

From the various columns tried, OV-1 on 100-200 mesh diatomile CQ treated gave the best resolution. The best concentration of stationary phase was found to be 3%. Decreasing the oven temperature and carrier gas flow rate increased resolution, but the N_2 flow rate was not decreased below 25ml/min by concerning the column efficiency.

Using the procedure described in the experimental section, N-TFA-L-prolyl-2-amino-1-butanol diastereoisomers were analyzed. As the result, the L-TP-*d*-2AB and L-TP-*l*-2AB diasteroisomers were separated with 98.43% resolution (R=1.125), with the former eluting typically in about 22.4 min. and the latter in about 20.6 min.

In hopes of the better resolution, converted the hydroxyl group of 2-AB to the less polar trimethyl silyl(TMS) ether before separation by reaction with a silylating agent such as TSIM. However, unexpectedly, the *d*-form and *l*-form of 2-AB were not separated.

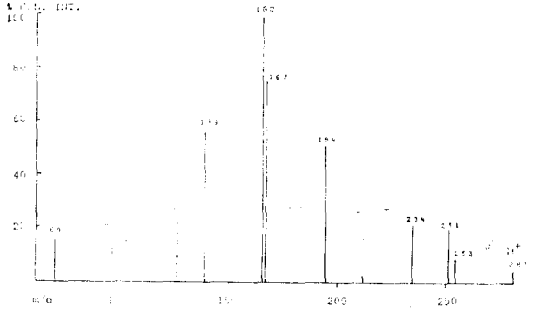


Fig. 2: Mass spectrum of TP-2AB
G. C Mass: Ael Model MS 1073 & Pye Unicam 104 G. C.
Column: 3%-OV-1 on Gaschrome Q 80/100 2ml long × 4mm i. d. 160°C isothermal
Carrier gas: He 27ml/min. G. C. Mass inter oven: 200°C
Electron energy: 70 eV. Sample size: 1μl.

Table III: Formal interpretation of the mass spectrum of TP-2AB.

m/e	ion fragment
282	M^+
253	$M-(C_2H_5)$
251	$M-CH_2OH$
194	$M-(C_2H_5$, CH_2OH and $CH_2=CH_2)$
166	$M-(NHCH(CH_2OH)C_2H_5$ and $CH_2=CH_2)$
139	$M-(COCF_3$, CH_2OH and $CH_3)$
126	$M-(COCF_3$, CH_2OH and $CH_2=CH_2)$
98	$M-(H_2C-N-COCF_3$, $CH_2=CH_2$ and $CH_2OH)$
69	CF_3^+

The retention times of L-TP-*d*-2AB-TMS and L-TP-*l*-2AB-TMS had a perfect equality.

The peaks of TP-2AB in the gas chromatogram were confirmed with the expected mass spectrum (molecular peak at m/e 282). (Fig. 2.)

The interpretation of the mass spectrum is following:

ETB

The TP derivatives of ETB could not be analyzed by G.L.C. The silylation of hydroxyl group of $(TP)_2$-ETB was necessary before injection, and when TSIM was used as a silylating reagent, the silylation reaction completes within 5 min at room temperature.[18~20] The trimethylsilylation must be done after the formation of TP derivatives of ETB was completed.

The free forms of ETB were obtained from their hydrochlorides by means of the chloroform extracting method which is used in the non-aqueous titration of ETB·2HCl.[21~22]

Table IV: Effect of the amount of the L-TPC on peak area ratio ($(TP)_2$-ETB-$(TMS)_2$).

L-TPC added	d-ETB/$C_{30}H_{62}$ (int. st.)
0.5ml	1.712
0.75	1.813
1.0	1.813
1.25	1.813

5 ml of *d*-ETB·2HCl (=3.7mg of *d*-ETB free base) trimethylamine: 0.1ml Reac. time: 15min TSIM: 200μl (reac. time: 5 min).

Table V: Effect of the amount of the Et_3N on peak area ratio ($(TP)_2$-ETB-$(TMS)_2$).

Et_3N added	d-ETB/$C_{30}H_{62}$ (int. st.)
0.1ml	1.813
0.2	1.813
0.3	1.813

5 mg of d-ETB · 2HCl (=3.7 mg of *d*-ETB free base) L-TPC: 1.0ml Reac. time: 15min TSIM: 200μl (reac. time: 5 min)

Evaluation of the optimun condition for the formation of $(TP)_2$-ETB with triethylamine and L-TPC was made by comparing peak area of *d*-ETB relate to that of n-$C_{30}H_{62}$. (Table IV and V)

As seen in Tables IV and V, when 0.75–1.0ml of TPC and 0.1 ml of Et_3N were added to 3.7 mg of ETB (=5mg of ETB·2HCl), the suitable peaks were obtained. Though the amounts of Et_3N and TSIM were increased in proportion to that of L-TPC, the peak area ratio didn't increase any more.

From the various columns tried, OV-1 on 80–100 mesh Shimalites W(AW-DMCS) (201D) gave the best resolution. The best concentration of stationary phase was found to be 1.5%.

The carrier gas flow rate was not decreased below 25 ml/min as the case of 2AB.

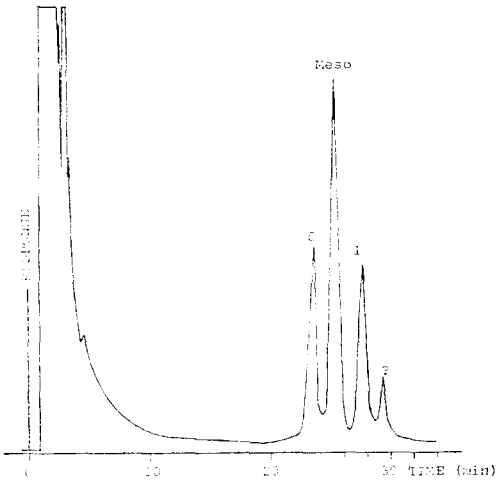


Fig. 3: Gas chromatogram of $(TP)_2$-ETB-$(TMS)_2$ diastereoisomers.
Instrument: Pye Unicam GCV chro.
Column: 2.1m long × 4mm i. d., glass with 1.5% OV-1 on shimalite W (AW-DMCS) (201D), 80–100 mesh, 240°C isothermal.
Injector: FID 280°C
Carrier gas: N_2 25ml/min
Sample size: 2μl

The ETB · 2HCl prepared from the unpurified racemic-2AB, containing 3–8 % of 1-amino-1-butanol (1-AB) as impurity, was extracted with chloroform to be free base form.

And using the procedure described in experimental section, $(N$-TFA-L-prolyl$)_2$-ETB-$(trimethylsilyl)_2$ diastereoisomers were analyzed by G.L.C. (Fig. 3). Under those conditions, the chromatogram produced for peaks instead of the expected three. And the molecular ion peak at m/e 734 could be seen in the mass spectrum of every four peaks.

The peak assignment was made using authentic samples of each of the ethambutol isomers in separate derivatizations with a exception of the fourth (the smallest) peak.

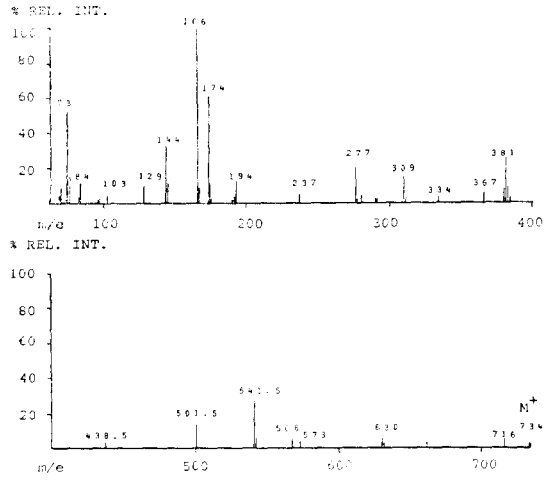


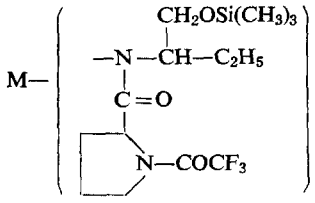
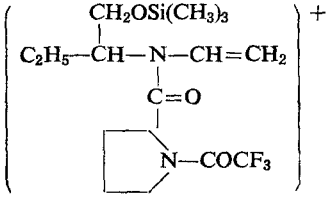
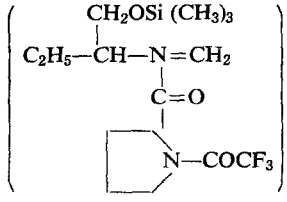
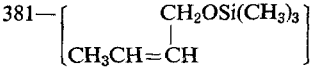
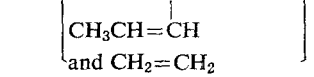
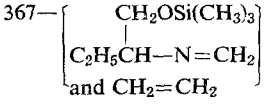
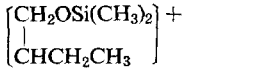
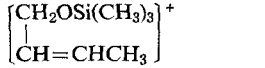
Fig. 4: Mass spectrum of $(TP)_2$–ETB–$(TMS)_2$
G. C. Mass: Shimadzu LKB-9000
Column: 1% OV-1 on Gaschrome Q 80/100
3m long × 4mm i. d. 240°C isothermal
Carrier gas: He 25ml/min.
G. C. Mass inter oven: 280°C.
Electron energy: 70 eV.
Sample size: 1 μl.

The first peak (R.T=23.6 min) was (L–TP)$_2$–*d*–ETB–$(TMS)_2$, the second (R.T=25.3 min) was (L–TP)$_2$–meso–ETB–$(TMS)_2$ and the third (R.T=27.6 min) was (L–TP)$_2$–*l*–ETB–$(TMS)_2$.

The fourth peak didn't appeared in the chromatogram produced by *d*–ETB, but almost always appeared in the chromatogram produced by *l*–ETB prepared from *l*–2AB containing 6–16 % of 1–AB because only *d*–2AB was picked out from the unpurified racemic–2–AB by glutamic acid or tartaric acid. The original substance producing the fourth peak seems to be a structural isomer–related with 1–AB–of ethambutol. $(TP)_2$–ETB–$(TMS)_2$ was confirmed with mass spectrum (Fig. 4).

Meso-form of ETB is removed easily from *d*–ETB by recrystallization, but *l*–form is not.

Table VI: Formal interpretation of the mass spectrum of $(TP)_2$–ETB–$(TMS)_2$.

m/e	ion fragment
734	M^+
716	$M-H_2O$
631	$M-(CH_2OSi(CH_3)_3)$
573	$631-(C_2H_5$ and $C_2H_5)$
381	
380	
367	
237	
194	
166	
145	
144	
103	$[CH_2OSI(CH_3)_3]^+$
73	$[Si(CH_3)_3]^+$
69	$[CF_3]^+$

Therefore they are the first (*d*-ETB) and the third (*l*-ETB) peaks that can be used for the management of quality. And as seen in Fig. 3, they were separated with baseline resolution.

LITERATURE CITED

1) Wilkinson, R. G. *et al.*, *J. A. C. S.* **83,** 2212 (1961).
2) Giorgio Z. C. A., **75,** 35119W
3) Gallardo, A., *ibid.* **74,** 87369b
4) Koenig, W. A. *et al.*, *J. Chromatog. Sci.* **8,** 183 (1970).
5) Nakaparksin, S. *et al.*, *ibid.* **8,** 177 (1970).
6) Corbin, J. A. *et. al.*, *Anal. Chem.* **42,** 974 (1970).
7) Dabrowiak, J. C. *et al.*, *ibid.* **43,** 791 (1971).
8) Karger, B. *et al.*, *ibid.* **39,** 228 (1967).
9) Bonner, W. A., *ibid.* **46,** 2104 (1974).
10) Pollock, G. E., *ibid.* **44,** 2368 (1972).
11) Raulin, F. *et al.*, *J. Chromatog.* **75,** 13 (1973).
12) Halpern, B. *et al.*, *Tetrahedron Letters* No. 21, 2238 (1966).
13) Bonner, W. A., *J. Chromatog. Sci.* **10,** 159 (1972).
14) Iwase, H. *et al.*, *Chem. Pharm. Bull.* **22,** 8(1974).
15) Goris, E., *Biochem. Pharmacol.* **15,** 2124 (1966).
16) Goris, E. *ibid.*, **16,** 863 (1967).
17) Beckett, A. H. *et al.*, *J. Pharm. Pharmc.* **25,** 382 (1973).
18) Richard, B. M. *et al. J. Chromatog.*, **87,** 80 (1974).
19) Calo, A. *et al.*, *ibid.* **37,** 194 (1968).
20) Lee, C. S. *et al. J. Pharmac. Sci.* **67,** 471 (1978)
21) U. S. P. XX: 304 p.
22) B. P. 1980: 178 p.
23) Knapp, D. R. *Handbook of Analytical Derivatization Reactions.* A Wiley-Interscience Pub. (1979). 85 p.
24) McNair H. M. and Bonelli E. J., *Basic Gas Chromatography,* Varian aerograpy (1969.)
25) McLafferty, F. W. *Interpretation of mass spectra,* second ed. Advanced Book Program (1973).