Asian Journal of Chemistry Vol. 19, No. 7 (2007), 5399-5406

Synthesis and Elucidation of 4-*bis*(2-Chloroethyl)-amino-L-phenylalanine

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In this research, synthesis of 4-*bis*(2-chloroethyl)amino-L-phenylalanine (melphalan) was carried out in several steps, starting with the amino acid, DL-phenylalanine. The structure of all the intermediates and the final product, melphalan, was determined by using various spectroscopic techniques.

Key Words: Melphalan, 4-*bis*(2-Chloroethyl)amino-L-phenylalanine, Alkeran, Phenylalanine mustard.

INTRODUCTION

Melphalan, (4-bis(2-chloroethyl)amino-L-phenylalanine) (**1**), is a nitrogen-mustard alkylating agent possessing two alkylating groups bind to cellular constituents through covalent bonds, which interferes with the growth of cancer cells and slows their growth and spread in the body.

Alkeran (melphalan), also known as L-phenylalanine mustard, phenylalanine mustard, L-PAM, or L-sarcolysin, is a phenylalanine derivative of nitrogen mustard. Melphalan is the active L-isomer of the compound and was first synthesized in 1953 by Bergel and Stock[1]. The D-isomer, known as medphalan, is less active against certain animal tumours and the dose needed to produce effects on chromosomes is larger than that required with the L-isomer. The racemic (DL-) form is known as merphalan or sarcolysin. Melphalan is practically insoluble in water and has a pK_{a1} of *ca*. 2.5. The molecular formula is $C_{13}H_{18}N_2O_2Cl_2$ and the molecular weight is 305.20. Melphalan is a bifunctional alkylating agent which is active against selective human neoplastic diseases. It is known chemically as 4-[*bis*(2-chloroethyl)amino]-L-phenylalanine. Melphalan is a cancer (antineoplastic) medication. Melphalan interferes with the growth of cancer cells and retards their growth and spread in the body. Melphalan is used to treat multiple myeloma (a type of blood cancer), cancer of the ovary and breast cancer. Alkylation of DNA within the nucleus of the cells is the

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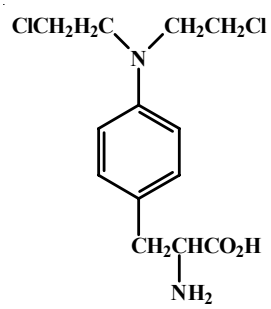
predominant reaction, which causes damage to the cells. Nitrogen atom at position 7 and oxygen atom at position 6 in the molecule of guanine are the most important sites for the alkylation of DNA (Fig. 1). Melphalan shows antitumor activity, possibly through binding DNA and RNA together and also through inhibiting protein synthesis. Melphalan has been used for the treatment of malignant melanoma, soft tissue sarcoma, neurobastoma, cancer of testis, non-Hodgkin's lymphoma and osterogenic sarcoma. Melphalan should only be administered under the supervision of a qualified healthcare provider experienced in the use of cancer chemotherapeutic agents. Serious side effects have been reported with the use of melphalan such as: allergic reactions (difficulty in breathing, chocking of throat; swelling of the lips, tongue, or face or hives); decreased bone marrow function and blood problems (extreme fatigue, easy bruising or bleeding, black, bloody



Melphalan, [4-*bis*(2-chloroethyl)amino-L-phenylalanine] (**1**)

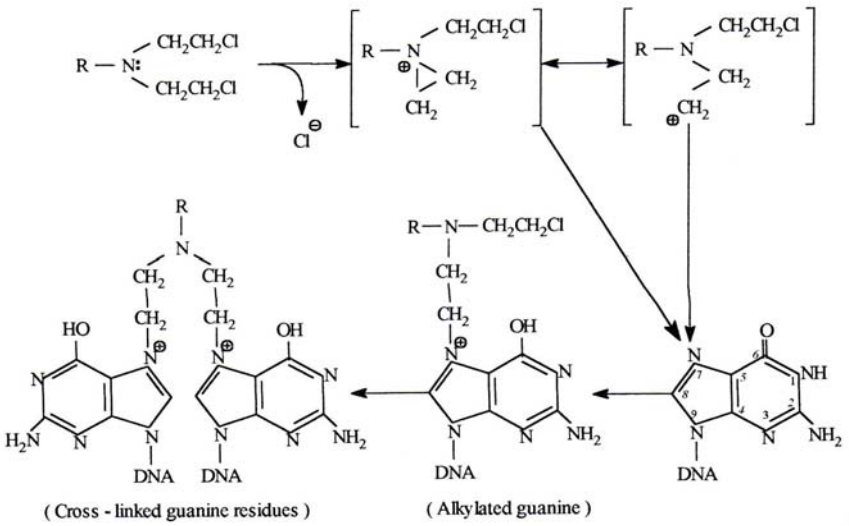


Fig. 1. Mechanism of alkylation of guanine DNA

or tarry stools, fever or chills, or signs of infection such as fever, chills, or sore throat), lung problems, severe nausea, vomiting, diarrhea and loss of appetite, *etc*. Therefore, a patient should talk to medical practitioner about the possible side effects from treatment with melphalan. In some cases, second cancers have been reported to occur during and following treatment with melphalan. A patient must discuss to medical practitioner about the risks and benefits of this medication. Before taking melphalan, a patient should talk to medical practitioner if he/she has (i) kidney disease or (ii) has bone marrow problems. One should not be able to take melphalan, or may require a dosage adjustment or special monitoring during treatment if has any of the conditions listed above[2].

EXPERIMENTAL

NMR spectra were recorded on a Bruker at 400 MHz (^{1}H) and on a Bruker at 80 MHz (^{1}H). Chemical shifts were measured in ppm on the δ scale downfield from TMS as internal standard. Mass spectra were measured on a Q70 Finniganmat mass spectrometer. Infrared spectra were recorded using a Jasco IR 700, as nujol mulls. UV spectra were recorded using a Jasco UV-Vis. 7850. All the chemicals were obtained from Merck.

Preparation of 4-nitro-DL-phenylalanine(II): To 10 mL concentrated nitric acid in a 100 mL Erlenmeyer flask which was cooled in an ice bath, 10 mL concentrated sulphuric acid was added in portions (2-3 mL each time) while stirring the mixture. (12.5 g, 75.75 mmol) DL-phenylalanine was added to the mixture in portions (2-3 g) and the mixture stirred vigorously until a homogeneous mixture was obtained[3], then heated at 100°C for 2-3 h. The mixture was poured in 500 mL water and crushed ice with vigorous stirring until a homogeneous solution was obtained. Concentrated ammonia was added to the homogeneous solution until it became alkaline, then heated to reduce the volume of the solution, a solid material appeared to form. The solution was cooled in an ice bath which resulted in the formation of a yellow precipitate. The product was filtered on a Buchner funnel and washed with cold water. Recrystallization with water resulted in the formation of a yellow solid material (14.31 g, 68. 18 mmol, 90 %), m.p. 237-240°C (literature[1] 235-240°C). Its IR spectrum (nujol mull) showed ν_{max} (cm^{-1}): 3282 (N-H, m)m 2924, 2854 (C-H, s), 1643-1620 (C=O, m-s), 1585, 1443 (C=C, aromatic ring, m,s), 1537, 1342 (N=O, s), 1282, 1198, 1105 (C-O, s) and 855 (C-$H_{bending}$, aromatic ring, s). ^{1}H NMR (D_2O + DCl, 80 MHz) showed (δ): 2.90-3.22 (m, CH_2 + NH_2), 3.50-3.80 (m, 1H) and 7.30-8.20 (AA'BB', 4 H). The mass spectrum (EI) showed m/z: 212 [$(M+2)^+$, 0.09 %)], 210 [$(M)^+$, 0.09%)], 164 [$(M-NO_2)^+$, 0.2 %)], 136 [$(M-CO_2HCH(NH_2)^+$, 0. 33 %)], 122 [$(M-CO_2HCH(NH_2)CH_2)^+$, 0.1 %)], 119

[(M-(CO_2H + NO^+))$^+$, 15.23 %)], 88 [(M-$C_6H_4NO_2$)$^+$, 55 %)], 74 [(M-CH_2 $C_6H_4NO_2$)$^+$, 100 %)] and 46 [(M-$CO_2HCH(NH_2)CH_2$ C_6H_4)$^+$, 12.4 %)].

Preparation of 4-nitro-N-phthaloyl-DL-phenylalanine (III): (10 g, 47. 62 mmol) of 4-nitro-DL-phenylalanine (**II**) was refluxed with phthalic anhydride (7. 05 g, 1 mmol) in dry pyridine (75 mL) for 2 h. A clear solution was obtained. The solution was concentrated by vacuum steam distillation and the residue refluxed for 15-20 min with acetic anhydride (50 mL), the cold liquid was poured into a mixture of water and ice (250 mL). The product was washed with cold water thoroughly and left it overnight at room temperature. The solid material was collected on a Buchner funnel and recrystallized from chloroform/petroleum ether (b.p. 40-60°C), a solid colourless material was obtained (22.16 g, 65.45 mmol, 96 %), m.p. 180-182°C) (lit.[1] 180-181°C). Its IR spectrum (nujol mull) showed ν_{max} (cm^{-1}): 2920, 2854 (C-H, s), 1772, 1707 (C=O, anhydride, m,s), 1607, 1465 (C=C, aromatic ring, m,s), 1524, 1345 (N=O, s), 1285, 1110 (C-O, s). ^{1}H NMR ($CDCl_3$, 400 MHz) showed (δ): 3.70 (d, 2H), 5.20 (t, 1H), 7.36-8.10 (AA'BB', 4 H) and 7.7-7.81 (m, 4H). The mass spectrum (EI) showed m/z: 295[(M-CO_2H)$^+$, 0. 01 %)], 194 [(M-C_6H_4 C_2O_2N)$^+$, 8.1 %], 147 {[M-(CH_2CH $CO_2HC_6H_4NO_2$+H)]$^+$, 4.8 %]}, 136 [(M-C_6H_4 $C_2O_2NCHCO_2H$)$^+$, 0.1 %)], 122[(M-C_6H_4 $C_2O_2NCHCO_2HCH_2$)$^+$, 0.2 %)], 104[(M-$NO_2C_6H_4CH_2CH(CO_2H)NCO$)$^+$, 54.3%)], 90[(M-($C_6H_4$ C_2O_2NCH CO_2H + NO_2)$^+$, 43.8%)], 76[(M-$NO_2C_6H_4CH_2CH(CO_2H)NC_2$ O_2)+, 100 %)] and 45[(M-$NO_2C_6H_4CH_2CHNC_2O_2$ C_6H_4)$^+$, 23.8 %)].

Preparation of 4-nitro-N-phthaloyl-DL-phenylalanine ethyl ester(IV): 100 mL dry ethanol was placed in a 250 mL 3-necked round bottomed flask equipped with a double surface reflux condenser. Dry gaseous HCl was passed through the alcohol until it became saturated *i.e.* there was an increase of 20 g in weight of the content of the flask (the round bottomed flask was placed in an ice bath). To this solution, (16.5 g, 48.53 mmol) of 4-nitro-N-phthaloyl-DL-phenylalanine (**III**) was added and refluxed for 4-6 h while dry HCl gas was passed through the solution continuously. The reaction mixture was cooled and the excess ethanol was removed *in vacuo*. The solid crystalline material was collected on a Buchner funnel. Recrystallization from *n*-propanol gave a colourless crystalline material (12.50 g, 33.98 mmol, 70 %), m.p. 95-97°C (literature[1] 96.5-97°C). Its IR spectrum (nujol mull) showed ν_{max} (cm^{-1}): 2922, 2852 (C-H, s), 1776, 1743, 1717 (C=O, anhydride, m,s), 1603, 1465 (C=C, aromatic ring, m,s), 1515, 1342 (N=O, s), 1241, 1199, 1105 (C-O, s). ^{1}H NMR ($CDCl_3$, 400 MHz) showed (δ): 1.26 (t, 3H), 3.68 (t, 2H), 4.26 (m, 2H), 5.26 (t, 1H), 7.36-8.08 (AA'BB', 4 H) and 7.69-7.81 (m, 4H). The mass spectrum (EI) showed m/z: 294[(M-$CH_3CH_2CO_2H$)$^+$, 45.2 %)], 247 [(M-C_6H_4 NO_2)$^+$, 15.2 %], 233 {[M-($CH_2C_6H_4NO_2$ + H)]$^+$, 66.7 %]}, 222 [(M- C_6H_4 C_2O_2N)$^+$, 6.7

%)], 158[(M-$CH_2C_6H_4NO_2$, $HCO_2C_2H_5)^+$, 100 %)], 146[(M-$NO_2C_6H_4$ $CH_2CH(CO_2C_2H_5)^+$, 26.7 %)], 136[(M-(C_6H_4 C_2O_2NCH $(CO_2C_2H_5)^+$, 0.09 %)], 122 [(M-C_6H_4 C_2 O_2NCH $(CO_2C_2H_5)$ $CH_2)^+$, 0.09 %)], 104 [(M-CON $CH(CO_2C_2H_5)$ $CH_2C_6H_4$ $NO_2)^+$, 48.6 %)] and 77 {[M-(C_2O_2N $CH(CO_2C_2H_5)$ $CH_2C_6H_4$ NO_2 + $H)^+$, 99 %)]}.

Preparation of 4-amino-N-phthaloyl-DL-phenylalanine ethylester hydrochloride (V): 4-Nitro-N-phthaloyl-DL-phenylalanine ethyl ester (**IV**) (9 g, 24.45 mmol) was hydrogenated at atmospheric pressure in ethyl acetate-methanol (50 mL each) over palladium-calcium carbonate (0.4 g). The solution mixture filtered and the solvent was evaporated on a rotary evaporator. A gummy material obtained which was dissolved in ether. Then, ethereal solution of HCl was added to the mixture while stirring vigorously. A gummy precipitate collected on a Buchner funnel which was washed with ether thoroughly. Recrystallization from ethyl acetate-acetone gave a needle crystalline material (8.52 g, 22.74 mmol, 93 %), m.p. 204-207°C (literature[1,4-6] 205-207°C). Its IR spectrum (nujol mull) showed ν_{max} (cm^{-1}): 3520, 3490 (N-H, m), 2966, 2932 (C-H, s), 1775, 1712 (C=O, anhydride, m,s), 1604, 1467 (C=C, aromatic ring, m,s), 1519 (N-H bending, s), 1242, 1109 and 1019 (C-O, s). ^{1}H NMR (d_6-acetone, 80 MHz) showed (δ): 1.25 (t, 3H), 2.5 (t, 2H), 3.45 (s, 2H), 4.22 (m, 2H), 5.18 (m, 1H), 7.38 -8.08 (AA'BB', 4 H) and 7.65-7.81 (m, 4H).

Preparation of 4-di(2-hydroxyethylamino)-N-phthaloyl-DL-phenylalanine ethyl ester (VI): 4-Amino-N-phthaloyl-DL-phenylalanine ethyl ester hydrochloride (**V**) (1 g, 2. 67 mmol) was suspended in water (12 mL) and glacial acetic acid was added with stirring until dissolution was complete (8 mL of acid was added). Ethylene oxide (2 mL) was added with shaking and the mixture kept for 24 h at room temperature. The clear yellow solution was poured into water (100 mL), a slight excess sodium bicarbonate carefully added with stirring, a gummy precipitate was obtained which was extracted with ethyl acetate and dried over $MgSO_4$. The solvent was evaporated on a rotary evaporator. The clear yellow gummy 4-di(2-hydroxyethylamino)-N-phthaloyl-DL-phenylalanine ethyl ester (**VI**) did not crystallize. This procedure was repeated until 6 g of the product (**VI**) was collected.

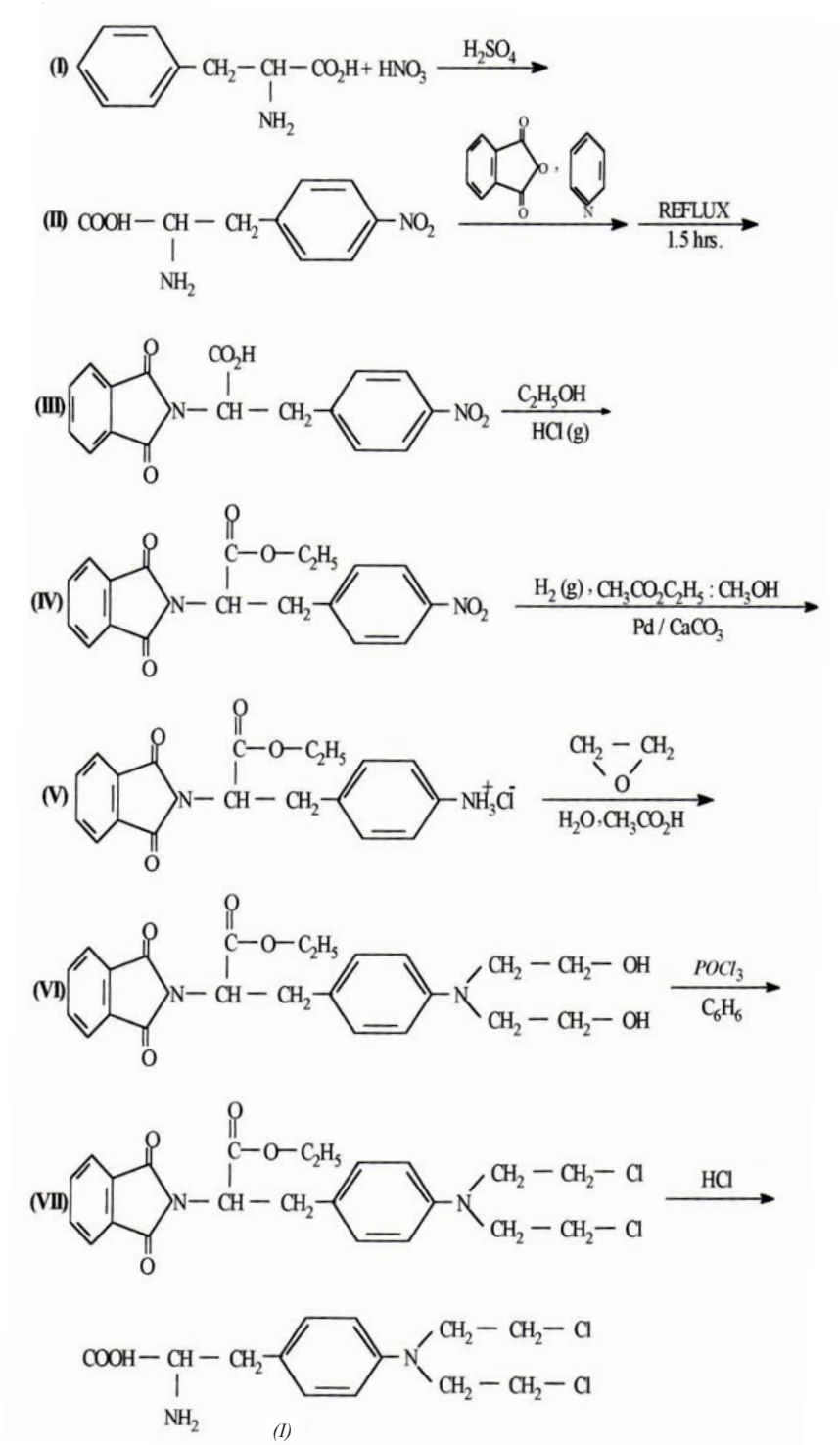
Preparation of 4-di(2-chloroethylamino)-DL-phenylalanine (I); (Melphalan): 4-Di(2-hydroxyethylamino)-N-phthaloyl-DL-phenylalanine ethyl ester (**VI**) (6 g, 16. 02 mmol) was dissolved in benzene (100 mL) and the solution was dried by distilling off part (25 mL) of the solvent. Freshly distilled phosphorus oxychloride (15 mL) was added and the mixture refluxed for 45 min. The solution was evaporated on a rotary evaporator until a clear gummy residue was obtained (**VII**). Concentrated hydrochloric acid was added to the gummy residue and refluxed for about 8 h. The

pink solution was filtered and evaporated to small bulk on a rotary evaporator. Addition of concentrated sodium acetate solution precipitated a white granular solid which crystallized from methanol as colourless needles (**I**) (2.25 g, 7.38 mmol, 45 %). It had m.p. 178-180°C (literature[5] 180-181°C). Its IR spectrum (nujol mull) showed ν_{max} (cm^{-1}): 3600-3100 (O-H, broad overlapped with N-H), 3000, 2850 (C-H, s), 1750 (C=O, m,s), 1600, 1450 (C=C, aromatic ring, m,s), 1540 (N-H bending, s), 1150 (C-N, m). The mass spectrum (EI) showed m/z: 231[$(M\text{-}NH_2CHCO_2H)^+$, 12.4 %)], 218 [$(M\text{-}(NH_2CH(CO_2H)CH_2 + H)^+$ 11.4 %], 164 [$M\text{-}N(CH_2CH_2Cl)_2^+$, 0.33 %], 118 [$(M\text{-}N(CH_2CH_2Cl)_2, HCO_2H]^+$, 5 %)], 104{[$M\text{-}(N(CH_2CH_2Cl)_2, CO_2H, NH_2)+ H]^+$, 51.4 %}, 90{$M\text{-}[NH_2CHCO_2H, N(CH_2CH_2Cl)_2]^+$, 53.3%}, 89 {$[M\text{-}(C_6H_4N(CH_2CH_2Cl)_2)+H]^+$, 60 %}, 76 {$M\text{-}[NH_2CH(CO_2H)CH_2, N(CH_2CH_2Cl)_2]^+$, 100 %}, 74 {$[M\text{-}(CH_2C_6H_4N(CH_2CH_2Cl)_2]^+$, 27 %}; its UV spectrum (5 mg in methanol) showed: λ_{max} (nm) 260 ($\log \varepsilon = 0.331$) and 301 ($\log \varepsilon = 0.015$).

RESULTS AND DISCUSSION

Developments in the field of tumor- or leukaemia-inhibiting agents reveal that α-amino acid derivatives play a crucial role in synthesizing such drugs. The reason behind this is due to a considerable extent, to the fact that such units occurred in the molecules of antibiotics and to the discovery of antimetabolite effects with the analogues of essential amino acids. As mentioned in the introduction part of this article melphalan is a cancer (antineoplastic) medication which interferes with the growth of cancer cells and retards their growth and spread in the body. Due to the significance of this drug, we prepared it in a multi step synthesize and characterized all the intermediates and the final product by using various spectra.

Synthesis of melphalan was carried out in several steps, starting with the amino acid, DL-phenylalanine. First, nitration of the benzene ring of DL-phenylalanine at the *p*-position was carried out. In the second step, in order to protect the amino group of *p*-nitro-DL-phenylalanine (**II**), the latter was treated with phthalic anhydride, converting the amino group into N-phthaloyl group (**III**). In the third step, the carboxyl group was converted to ethyl ester through esterification of *p*-nitro-N-phthaloyl-DL-phenylalanine with excess ethanol (**IV**). In the fourth step, catalytic hydrogenation of *p*-nitro-N-phthaloyl-DL-phenylalanine ethyl ester (**IV**), gave *p*-amino-N-phthaloyl-DL-phenylalanine ethyl ester which was subsequently converted to its hydrochloride salt (**V**). In the fifth step, the hydrochloride salt was treated with ethylene oxide in glacial acetic acid which resulted in


(I)
H2SO4
(II)
REFLUX
1.5 hrs.
(III)
C2H5OH
HCl (g)
(IV)
H2 (g), CH3CO2C2H5 : CH3OH
Pd / CaCO3
(V)
H2O, CH3CO2H
(VI)
POCl3
C6H6
(VII)
HCl
(I)

Scheme-I

the formation of (**VI**). In the sixth step, treatment of (**VI**) with phosphorous oxychloride, resulted in the formation of *p*-di(2-chloroethylamino)-N-phthaloyl-DL-phenylalanine ethyl ester (**VII**). Finally, acidic hydrolysis of the latter compound with hydrochloric acid gave melphalan (**I**) as a white crystalline powder (**Scheme-I**). All the yields were quite good and acceptable.

REFERENCES

1. F. Bergel and J.A. Stock, *J. Chem. Soc.*, 2409 (1954).
2. http://health.yahoo.com/drug/d00287a1, Accessed 8th of Oct. 2006.
3. C.R. Craig and R.E. Stitzel, Modern Pharmacology, Little Brown and Company, Boston, edn. 3, pp. 776-821 (1990).
4. F. Bergel, V.C.E. Burnop and J.A. Stock, *J. Chem. Soc.*, 1223 (1955).
5. C. Dollery, Therapeutic Drugs, Churchill Livingstone, London, Vol. 1, pp. 48-51 (1991).
6. M. Windols, The Merck Index, Merck and Co. Inc., NJ, USA, edn. 19, p. 914 (1976).

(*Received*: 18 October 2006; *Accepted*: 18 June 2007) AJC-5716