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Synthesis and Uncoupling Activities of Hydrophobic Thioureas

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Various *N*-aryl-*N'*-phenylthioureas, *N*,*N'*-diarylthioureas and *N*-(1,2,4-triazol-3-yl)-*N'*-arylthioureas were prepared and examined for uncoupling activities. The results indicate that substitution at the 4-position of the phenyl groups of diaryl thioureas is very important for uncoupling activities. Diphenyl thioureas substituted with two or more halogen atoms exhibited strong activities. The highest activity was exhibited by a compound containing nitro groups on both phenyl groups. These results indicate that the hydrophobicity and acidic nature of the compound are of primary importance for uncoupling activities. A remarkable decrease in activity was observed with the thioureas which were substituted with pyridine and 1,2,4-triazole rings. The reaction of phenyl isothiocyanate with 3-amino-1,2,4-triazole was also studied.

Keywords——uncoupling activity; oxidative phosphorylation; mitochondria; *N*,*N'*-diarylthiourea; *N*-(1,2,4-triazol-3-yl)-*N'*-arylthiourea; 3-amino-1,2,4-triazole; aryl isothiocyanate; *N*,*N'*-bis(1,2,4-triazol-3-yl)thiourea

Recently, we reported that nonyl 3-acyldithiocarbazates act as effective uncouplers of oxidative phosphorylation in mitochondria, and the presence of thiocarbamoyl structure with the potential SH group is essential for their uncoupling activities.[1-4] They act as protonophoric uncouplers rather than "direct reactive" uncouplers.

Hydrophobic isothiocyanates, such as 4-bromophenyl isothiocyanates, have been reported as a new class of uncouplers, because such compounds have no dissociable proton.[5] However, our previous results showed that 4-bromophenyl isothiocyanate is readily transformed into *N*,*N'*-bis(4-bromophenyl)thiourea in dimethyl sulfoxide (DMSO), which was used as a solvent for the concentrated stock solution of the isothiocyanate, and the resulting thiourea derivative (possessing a potential SH group) is active.[6] *N*,*N'*-Disubstituted thioureas are known to exhibit antiviral,[7] antituberculous,[8] fungicidal,[9] and herbicidal[10] activities. In addition, *N*-monoalkyl thioureas and 6-alkyl-2-thiouracils have been reported to exhibit another type of inhibition of oxidative phosphorylation.[11]

These findings prompted us to investigate the effects of various aromatic thioureas on oxidative phosphorylation in mitochondria. This paper describes the synthesis of *N*-aryl-*N'*-phenylthioureas, *N*,*N'*-diarylthioureas, and *N*-(1,2,4-triazol-3-yl)-*N'*-arylthioureas and the determination of their uncoupling activities.

Thioureas **1**—**15** were prepared by condensation of aromatic amines with substituted arylisothiocyanates. The reaction of phenylisothiocyanate with 3-amino-1,2,4-triazole gave two products, *N*-(1,2,4-triazol-3-yl)-*N'*-phenylthiourea (**16**) and 5-amino-1-[phenylamino-(thiocarbonyl)]-1,2,4-triazole (**20**). The structures of **16** and **20** were determined on the basis of the nuclear magnetic resonance (NMR) and mass spectroscopic data. The NMR spectrum of **20** showed the signals of C_3-H at δ 7.68 and NH_2 at δ 8.25 with an NH proton at δ 11.45 as a broad absorption, while that of **16** showed the C_5-H at δ 8.46 and three NH pro-

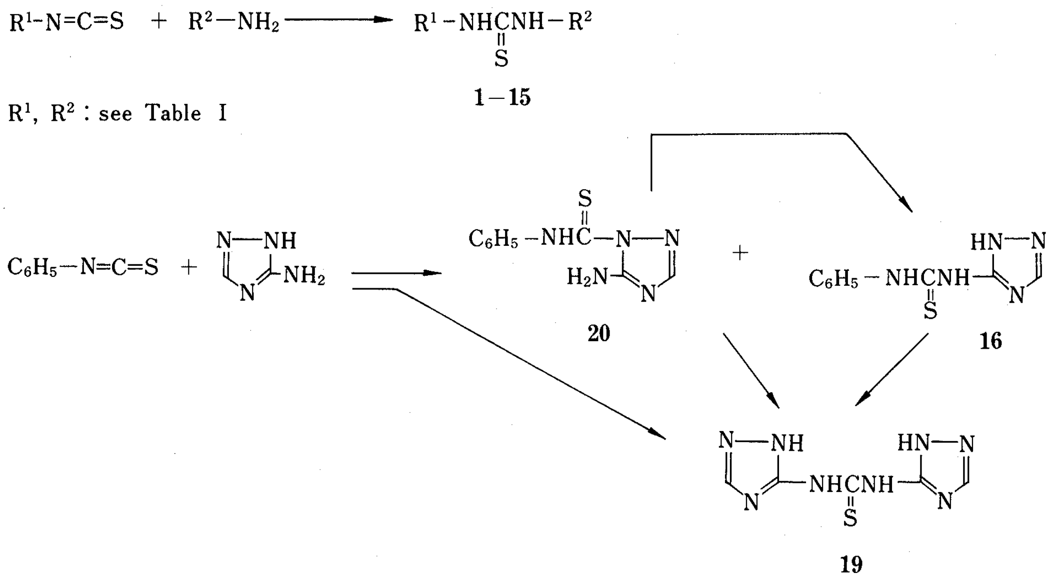

Chart 1

tons at δ 11.20, 11.84, and 13.40 as very broad absorptions. These spectroscopic data are in good agreement with those of 5-amino-1-[methylamino(thiocarbonyl)]-1,2,4-triazole and *N*-(1,2,4-triazol-3-yl)-*N'*-methylthiourea, respectively.[12] The fragmentation patterns in the mass spectra (MS) of **16** and **20** gave additional support for the proposed structures. The mass spectrum of **16** showed a molecular ion peak at *m/z* 219 and characteristic fragments at *m/z* 185 ($M^+ - H_2S$), and 84 ($M^+ - C_6H_5NCS$), with strong peaks of *m/z* 93 ($C_6H_5NH_2$) and 135 (C_6H_5NCS), while that of compound **20** showed fragments at *m/z* 135 (C_5H_5NCS), 84 (aminotriazole), and 77 (benzene) with a molecular ion peak at *m/z* 219.

The ratio of the amount of product **16** to that of **20** was found to depend on the reaction conditions. For example, the reaction of phenyl isothiocyanates with 3-amino-1,2,4-triazole in dry acetone at room temperature gave **16** and **20** in 2 and 56% yields, respectively. When the reaction was performed in the absence of solvent at 100 °C for 3 h, **20** (59%) was obtained as the major product and **16** (8%) as a minor product. However, the same reaction in dimethylformamide (DMF) at room temperature gave **16** and **20** in 53 and 13% yields, respectively. Therefore, we used DMF as a solvent for the synthesis of compounds **16—18**.

Compound **16** or **20**, when heated in *o*-dichlorobenzene, was found to be converted to *N,N'*-bis(1,2,4-triazol-3-yl)thiourea (**19**) in 56 or 88% yield, respectively. The thiourea (**19**) was also obtained in 30% yield by heating a mixture of 3-amino-1,2,4-triazole and phenyl isothiocyanate in *o*-dichlorobenzene.

Uncoupling Activity

We have reported that *N,N'*-bis(4-bromophenyl)thiourea (BBTU, **10**) at 10 μM completely released oligomycin-inhibited respiration with either glutamate plus malate, or succinate as a substrate, and it activated adenosine triphosphatase (ATPase) in mitochondria.[6] This compound has protonophoric activity, as observed with other commonly used weakly acidic uncouplers. These data indicate that BBTU (**10**) is a typical weakly acidic uncoupler of oxidative phosphorylation in mitochondria.

In the present study, the uncoupling activities of all thioureas were determined by measuring changes in the state 4 respiration of rat liver mitochondria using succinate (plus rotenone) as a substrate. The uncoupling activities of various thioureas (**1—20**) are shown as the concentrations (μM) inducing maximal release of state 4 respiration. The relative activities listed in Tables I and II are those relative to the activity of BBTU (**10**).

TABLE I. Uncoupling Activities of *N*, *N'*-Diarylthioureas

R^1–NHC(=S)NH–R^2

No.	R^1	R^2	mp[a] (°C)	Uncoupling[b] activity (μM)	Relative[c] activity (%)
1	Ph	Ph	150—151[d]	683.0	1.6
2	Ph	4-CH_3Ph	149—151[e]	585.0	1.9
3	Ph	4-CH_3OPh	149—150[f]	895.0	1.2
4	Ph	4-ClPh	155—156[g]	66.0	16.7
5	Ph	4-BrPh	155—156[h]	64.0	17.2
6	Ph	4-NO_2Ph	146—148[i]	20.0	55.0
7	Ph	3,4-Cl_2Ph	95—97	10.0	110.0
8	4-ClPh	4-ClPh	174—175[j]	11.0	100.0
9	4-BrPh	4-ClPh	179—181	13.0	84.6
10	4-BrPh	4-BrPh	183—184[k]	11.0	100.0
11	4-BrPh	3,4-Cl_2Ph	159—161	3.6	305.6
12	4-NO_2Ph	4-NO_2Ph	195—196[l]	1.2	916.7
13	4-BrPh	4-CH_3OPh	183—184	Inactive	0.0
14	Ph	2-Pyridyl	166—169[m]	Inactive	0.0
15	Ph	4-Pyridyl	143—144[n]	Inactive	0.0

a) All compounds gave satisfactory C, H, N elementary analyses. *b*) The concentration of the compound inducing maximal release of state 4 respiration of mitochondria. *c*) Relative activity (percent) with respect to **10**. *d*) Ref. 18: mp 154—155 °C. *e*) Ref. 18: mp 140—142 °C. *f*) Ref. 18: mp 151—153 °C. *g*) Ref. 18: mp 154—155 °C. *h*) Ref. 19: mp 158 °C. *i*) Ref. 18: mp 150—152 °C. *j*) Ref. 20: mp 176 °C. *k*) Ref. 21: mp 184—185 °C. *l*) MS *m/z* 284 ($M^+ - H_2S$). Ref. 18: mp 210—212 °C. *m*) Ref. 22: mp 167—168 °C. *n*) Ref. 23: mp 148 °C.

TABLE II. Uncoupling Activities of 1,2,4-Triazolylthiourea

R^1–NHC(=S)–R^2

No.	R^1	R^2	mp[a] (°C)	Uncoupling[b] activity (μM)	Relative[c] activity (%)
16	Ph	3-NHTri[d]	208—210	Inactive	0.0
17	4-ClPh	3-NHTri	209—211	115	9.6
18	4-BrPh	3-NHTri	212—214	87	12.6
19	3-Tri[e]	3-NHTri	300	Inactive	0.0
20	Ph	2-(3-NH_2Tri)[f]	139 (dec.)	Inactive	0.0

a) All compounds gave satisfactory C, H, N elementary analyses. *b*) The concentration of the compound inducing maximal release of state 4 respiration of mitochondria. *c*) Relative activity (percent) with respect to **10**. *d*) 3-Imino-1,2,4-triazolyl. *e*) 3-(1,2,4-Triazolyl). *f*) 2-(3-Amino-1,2,4-triazolyl).

These data suggest that substitution at the 4-position of the phenyl groups of diarylthioureas is very important for the uncoupling activity (Table I). Among mono-substituted derivatives (**2**—**6**) at the 4-position in a phenyl group of **1**, the introduction of a methyl or methoxy group did not affect the activity, while the introduction of a chloro or bromo group increased the activity about ten times. Furthermore, compounds with two or more halogen atoms (**7**—**9**) exhibited strong uncoupling activities, almost equal to that of BBTU (**10**). *N*-(4-Bromophenyl)-*N'*-(3,4-dichlorophenyl)thiourea (**11**) was three times more active than BBTU. The introduction of the electron-withdrawing group –NO_2 at the 4-positions of the two phenyl rings resulted in the strongest uncoupling activity. The activity of this compound (**12**) is more than nine times that of BBTU (**10**) and about forty times that of the most commonly used uncoupler 2,4-dinitrophenol.[13] The high potency may arise because of the symmetry of the molecule and because the nitro groups which are present on both phenyl rings increase the acidic nature of the molecule. Therefore, it appears that the acidity and the hydrophobicity due to the two phenyl rings are of primary importance for the uncoupling activities of thioureas. This view was supported by the fact that the substitution of a more hydrophilic pyridyl group such as 2-pyridyl (**14**) and 4-pyridyl (**15**), for a phenyl group caused a remarkable decrease or complete loss of uncoupling activities.

3-Amino-1,2,4-triazole is a commercial defoliant. We synthesized several thioureas (**16**—**20**) containing a 1,2,4-triazole ring and examined their uncoupling activities (Table II). However, they were found to be almost ineffective.

Experimental

Uncoupling Activity——Rat liver mitochondria were isolated according to the method of Hogeboom[14] as described by Myers and Slater.[15] The protein concentration of mitochondria was determined by the biuret method.[16] For determination of the uncoupling activity of a test compound, a suspension of state 4 mitochondria energized with 10 mM succinate (plus rotenone at 1 μg/mg protein) in an incubation medium consisting of 200 mM sucrose, 2 mM $MgCl_2$, 1 mM ethylenediamine tetraacetic acid (EDTA), and 10 mM phosphate buffer, pH 7.2, at 25 °C was titrated with a solution of the compound in DMSO. Respiration was measured in terms of oxygen uptake by mitochondria with a Clark oxygen electrode. The total volume of the reaction mixture was 3.3 ml, unless otherwise noted. The amount of mitochondria was about 0.7 mg protein/ml. The respiration was stimulated almost linearly over low concentration ranges of the compounds, but on further addition of the compounds, the respiration rate gradually reached a maximum level. The minimum concentration causing the maximal release of respiration was taken as 100% uncoupling activity.[17]

Synthesis——Melting points were determined by the capillary method and are uncorrected. NMR spectra were recorded on a JEOL PS-100 spectrometer using tetramethylsilane as an internal standard. Mass spectra were measured with a JEOL D-300 instrument. For column chromatography, a 1 : 1 mixture of Merck Silica gel 60 (70—230 mesh) and Mallinckrodt silicic acid (100 mesh) was employed.

***N*,*N′*-Diarylthioureas (1—15)**——These thioureas were prepared by the following method. A solution of an appropriate arylamine (4 mmol) in dry acetone (7 ml) was mixed with the corresponding aryl isothiocyanate (4 mmol) and the mixture was heated under reflux for several hours. The resulting precipitate was collected by filtration and crystallized from ethanol: **7** (yield 81% from 3,4-dichloroaniline), *Anal.* Calcd for $C_{13}H_{10}Cl_2N_2S$: C, 52.54; H, 3.39; N, 9.43. Found: C, 52.84; H, 3.43; N, 9.57. **9** (yield 85% from 4-chloroaniline), *Anal.* Calcd for $C_{13}H_{10}BrClN_2S$: C, 45.70; H, 2.95; N, 8.20. Found: C, 45.82; H, 2.89; N, 8.23. **11** (yield 63% from 3,4-dichloroaniline), *Anal.* Calcd for $C_{13}H_9BrClN_2S$: C, 41.52; H, 2.41; N, 7.45. Found: C, 41.40; H, 2.39; N, 7.54. **13** (yield 93% from anisole), *Anal.* Calcd for $C_{14}H_{13}BrN_2OS$: C, 49.86; H, 3.89; N, 8.31. Found: C, 49.80; H, 3.91; N, 8.45.

***N*-(1,2,4-Triazol-3-yl)-*N′*-phenylthiourea (16) and 5-Amino-1-[phenylamino(thiocarbonyl)]-1,2,4-triazole (20)**——Method A: A mixture of 3-amino-1,2,4-triazole (0.3 g, 3.6 mmol) and phenyl isothiocyanate (0.483 g, 3.6 mmol) in dry acetone (5 ml) was stirred at room temperature for 24 h. The resulting precipitate was filtered and recrystallized from methanol to give 0.275 g (35%) of **20**, mp 139 °C (dec.). ^{1}H-NMR (Me_2SO-d_6) δ: 7.68 (1H, s, C_3-H), 7.15—7.60 (5H, m, ArH), 8.25 (2H, br, NH_2), 11.45 (1H, br s, NH). MS *m/z*: 219 (M^+), 135, 84, 77. *Anal.* Calcd for $C_9H_9N_5S$: C, 49.30; H, 4.14; N, 31.94. Found: C, 49.18; H, 4.22; N, 32.06. The filtrate was chromatographed on a silica gel column (chloroform–acetone, 7 : 1, v/v). Evaporation of the first fraction gave a solid, which was crystallized from methanol to give 0.162 g (21%) of **20**. Evaporation of the second fraction gave a solid, which was crystallized from methanol to give 0.017 g (2%) of **16**, mp 208—210 °C. ^{1}H-NMR (Me_2SO-d_6) δ: 8.46 (1H, s, C_5-H), 7.05—7.70 (5H, m, ArH), 11.20 (1H, br s, NH), 11.84 (1H, br s, NH), 13.40 (1H, br s, NH). MS *m/z*: 219 (M^+), 185, 135, 126. *Anal.* Calcd for $C_9H_9N_5S$: C, 49.30; H, 4.14; N, 31.94. Found: C, 49.18; H, 4.14; N, 31.78.

Method B: A mixture of 3-amino-1,2,4-triazole (0.3 g, 3.6 mmol) and phenyl isothiocyanate (0.483 g, 3.6 mmol) was heated at 100 °C for 3 h. The same treatment as described in method A gave **16** (0.06 g, 8%) and **20** (0.463 g, 59%).

Method C: A mixture of 3-amino-1,2,4-triazole (0.3 g, 3.6 mmol) and phenyl isothiocyanate (0.483 g, 3.6 mmol) in DMF (2 ml) was stirred at room temperature for 24 h. The mixture was poured into ice-water, and the resulting precipitate was collected by filtration and chromatographed on a silica gel column (chloroform–acetone, 7 : 1, v/v). Evaporation of the first fraction gave a solid, which was crystallized from methanol to give **20** (0.103 g, 13%). Evaporation of the second fraction gave a solid, which was crystallized from methanol to give **16** (0.414 g, 53%).

Rearrangement to 20 to 16——Compound **20** (0.1 g, 0.456 mmol) was heated at 150 °C for 20 min, and the resulting solid was chromatographed on a silica gel column (chloroform–acetone, 7 : 1, v/v). Evaporation of the first fraction gave a solid, which was crystallized from benzene to give **1** (0.011 g, 21%). Evaporation of the second fraction gave a solid, which was crystallized from methanol to give **16** (0.06 g, 60%). Evaporation of the third fraction gave a solid, which was crystallized from EtOH–$CHCl_3$ to give *N*,*N*′-bis(1,2,4-triazol-3-yl)thiourea (**19**) as colorless needles (0.008 g, 17%), mp 300 °C. ^{1}H-NMR (Me_2SO-d_6) δ: 8.34 (2H, br s, C_5-H), 10—15 (4H, br, NH). MS *m/z*: 210 (M^+), 208, 126, 84, 70. *Anal.* Calcd for $C_5H_6N_8S$: C, 28.57; H, 2.88; N, 53.30. Found: C, 28.65; H, 2.90; N, 53.55.

***N*-(1,2,4-Triazol-3-yl)-*N*′-arylthioureas (17 and 18)**——Compounds **17** and **18** were prepared by the following method. A solution of 3-amino-1,2,4-triazole (1.334 g, 16 mmol) and the corresponding 4-substituted phenyl isothiocyanate (16 mmol) in DMF (10 ml) was stirred at room temperature for 24 h. Addition of water gave a precipitate, which was collected by filtration and crystallized from methanol: *N*-(1,2,4-triazol-3-yl)-*N*′-(4-chlorophenyl)thiourea (**17**) (3.53 g, 87%), mp 209—211 °C. ^{1}H-NMR (Me_2SO-d_6) δ: 7.40 (2H, d, ArH), 7.66 (2H, d, ArH), 8.48 (1H, s, C_5-H), 11.20 (1H, br s, NH), 11.76 (1H, br s, NH), 13.40 (1H, br s, NH). MS *m/z*: 253 (M^+), 219 ($M^+ - H_2S$). *Anal.* Calcd for $C_9H_8ClN_5S$: C, 42.61; H, 3.18; N, 27.60. Found: C, 42.69; H, 3.16; N, 27.28. *N*-(1,2,4-triazol-3-yl)-*N*′-(4-bromophenyl)thiourea (**18**) (3.96 g, 83%), mp 212—214 °C. ^{1}H-NMR (Me_2SO-d_6) δ: 7.60 (4H, s, ArH), 8.48 (1H, s, C_5-H), 11.80 (2H, br s, NH), 13.40 (1H, br s, NH). MS *m/z*: 297 and 299 ($M^+ - 1$ ^{79}Br and $M^+ + 1$ ^{81}Br). *Anal.* Calcd for $C_9H_8BrN_5S$: C, 36.25; H, 2.70; N, 23.49. Found: C, 36.30; H, 2.70; N, 23.56.

***N*,*N*′-Bis(1,2,4-triazol-3-yl)thiourea (19)**——Method A: A solution of **16** (0.1 g, 0.456 mmol) in *o*-dichlorobenzene (1 ml) was refluxed for 4 h, then allowed to cool. The precipitate was collected by filtration and crystallized from EtOH–$CHCl_3$ to provide colorless needles (0.027 g, 56%). This product was identical with **19** obtained by rearrangement of **20**.

Method B: Compound **20** (0.09 g, 0.41 mmol) was heated at 150—200 °C for 40 min, and then allowed to stand at room temperature. The resulting solid was crystallized from methanol to give **19** (0.038 g, 88%).

Method C: A mixture of 3-amino-1,2,4-triazole (5 g, 60 mmol) and phenyl isothiocyanate (8.03 g, 60 mmol) was heated at 100—110 °C for 30 min. The resulting solid was collected by filtration, washed with $CHCl_3$–MeOH and heated in *o*-dichlorobenzene (14 ml) at 130 °C for 14 h. The resulting precipitate was collected by filtration and crystallized from methanol to give **19** (1.92 g, 30%).

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References

1) H. Terada, M. Uda, T. Okitsu, F. Kametani, and S. Kubota, *FEBS Lett.*, **78**, 77 (1977).
2) S. Kubota, M. Uda, F. Kametani, and H. Terada, *J. Med. Chem.*, **21**, 591 (1978).
3) H. Terada, M. Uda, F. Kametani, and S. Kubota, *Biochim. Biophys. Acta*, **504**, 237 (1978).
4) M. Uda, K. Toyooka, K. Horie, M. Shibuya, S. Kubota and H. Terada, *J. Med. Chem.*, **25**, 557 (1982).
5) M. Miko and B. Chance, *Biochim. Biophys. Acta*, **396**, 165 (1975).
6) H. Terada and S. Kubota, *FEBS Lett.*, **100**, 37 (1979).
7) A. S. Galabov, B. S. Galabov and N. A. Neykova, *J. Med. Chem.*, **23**, 1048 (1980).
8) L. Doub, L. M. Richardson, D. R. Herbst, M. L. Black, O. L. Stevenson, L. L. Bambas, G. P. Youmans, and A. S. Youmans, *J. Am. Chem. Soc.*, **80**, 2205 (1958); A. C. Glasser and R. M. Doughty, *J. Pharm. Sci.*, **51**, 1031 (1962).
9) G. Vasilev and Z. Tomaleva, *Arch. Phytopathol. Pflanzenshuts*, **9**, 309 (1973); G. Krause, R. Franke, and G. N. Vasilev, *Biochem. Physiol. Pflanz*, **174**, 128 (1979).
10) G. Vasilev, L. Iliev, and R. Vasilev, *Mekh Deistviya Gerbits*, **1971**, 187 [*Chem. Abstr.*, **86**, 5117*d* (1977)].
11) E. Bäuerlein and R. Keihl, *FEBS Lett.*, **61**, 68 (1976).
12) T. Hirata, L. M. Twanmoh, H. B. Wood, Jr., A. Goldin, and J. S. Driscoll, *J. Heterocycl. Chem.*, **9**, 99 (1972).
13) H. Terada, *Biochim. Biophys. Acta*, **639**, 225 (1981).
14) G. H. Hogeboom, *Methods Enzymol.*, **1**, 16 (1955).
15) D. K. Myers and E. C. Slater, *Biochem. J.*, **67**, 558 (1957).
16) A. G. Gornall, C. J. Bardawill, and M. M. David, *J. Biol. Chem.*, **177**, 751 (1949).
17) H. Terada and K. van Dam, *Biochim. Biophys. Acta*, **387**, 507 (1975).
18) T. A. Briody, A. F. Hegarty, and F. L. Scott, *Tetrahedron*, **33**, 1469 (1977).
19) W. G. Finnegan, R. A. Henry, and E. Lieber, *J. Org. Chem.*, **18**, 779 (1953).
20) G. M. Dyson and H. J. George, *J. Chem. Soc.*, **1924**, 1704.
21) R. F. Hunter and C. Soyka, *J. Chem. Soc.*, **1926**, 2958.
22) A. E. S. Fairfull and D. A. Peak, *J. Chem. Soc.*, **1955**, 796.
23) R. Camps, *Arch. Pharm.*, **240**, 345 (1902).