Tetrahedron Vol. 32, pp 2071 to 2074 Pergamon Press 1976. Printed in Great Britain

OPTICALLY ACTIVE 4-SUBSTITUTED *cis*-1,2-DIPHENYLETHYLENE OXIDES AND RELATED 1,2-DIPHENYLETHANE DIOLS

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(*Received in the USA 6 January 1976; Received in the UK for publication 23 March 1976*)

Abstract—*Erythro* 1-(4'-chlorophenyl)- and 1-(4'-methylphenyl)-2-phenylethane diols with [1*S*:2*R*] and [1*R*:2*S*] absolute stereochemistry were synthesized and converted to the corresponding 4'-substituted [1*S*:2*R*] and [1*R*:2*S*] *cis*-stilbene oxides by the following sequence: formation of 2-methoxydioxolanes, conversion to halohydrins and cyclization to *cis* epoxides. Substantial amounts of optically active 4'-substituted *trans*-stilbene oxides were also produced. The enzyme epoxide hydrase stereoselectively adds water at the [*S*] carbon atom of the *cis*-stilbene oxides with inversion of configuration to produce [*R*:*R*] diols. Facile enzymatic resolution of *cis* - 1 - (4' - nitrophenyl) - 2 - phenylethylene oxide was achieved. Optical properties, including CD spectra of the above compounds, are described.

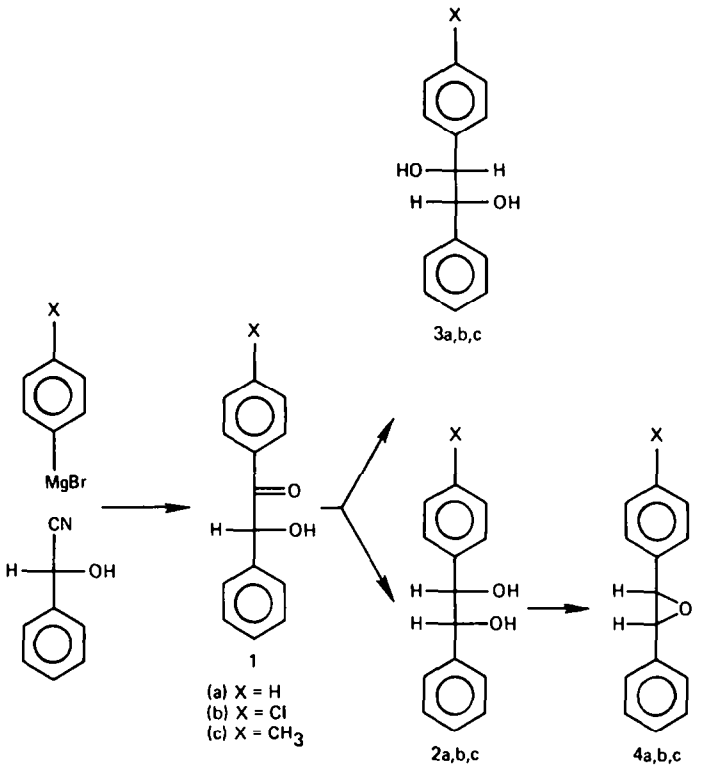
Many aromatic hydrocarbons and olefinic compounds are metabolized by mammals to vicinal diols via hydration of initially formed arene oxides or epoxides.[1,2] Epoxide hydrase, the enzyme responsible for this trans addition of water,[1,3-6] has been purified from several species[7-9] and may be important in the detoxication of cytotoxic and potentially carcinogenic metabolites.[10,11] The enzyme is regiospecific in the addition of water at the C-2 position of naphthalene 1,2-oxide[3,12] and C-8 of styrene oxide.[4] The stereospecificity of the hydration can vary in that racemic diol is formed from styrene oxide[4] while optically pure diol is formed from *cis*-stilbene oxide.[5,6]

Since *cis*-stilbene oxide is a good substrate for epoxide hydrase and since only the [*R*,*R*] *threo* diol is produced,[5,6] substituted stilbene oxides should be useful substrates to study the stereospecificity of the hydration. The required optically active 4' - substituted *cis*-stilbene oxides were obtained from optically active diols by the method of Newman[13] in which the diols are converted to epoxides with retention of configuration via halohydrins prepared from methoxydioxolanes. The resulting optically active *cis* epoxides [*S*,*R* or *R*,*S*] could be converted by epoxide hydrase to either [*R*,*R*] or [*S*,*S*] *threo* diols depending on which C atom of the epoxide is attacked. Racemic epoxides cannot be employed since, at low substrate conversion, it would be impossible to deduce which enantiomer had led to the observed product. Correlation of the CD spectra of the resultant diols with that of (+)-[1*R*,2*R*] - diphenylethane diol[14] would allow assignment of their absolute stereochemistry and thereby establish which C atom of the oxirane ring was preferentially attacked. The present study describes the synthesis of 4'-methyl and chloro-substituted *erythro* diphenylethane diols and the corresponding *cis*-diphenylethylene oxides as well as assigns their absolute stereochemistry. The optical properties of the above and of the enzymatically produced diols from the epoxides are discussed.

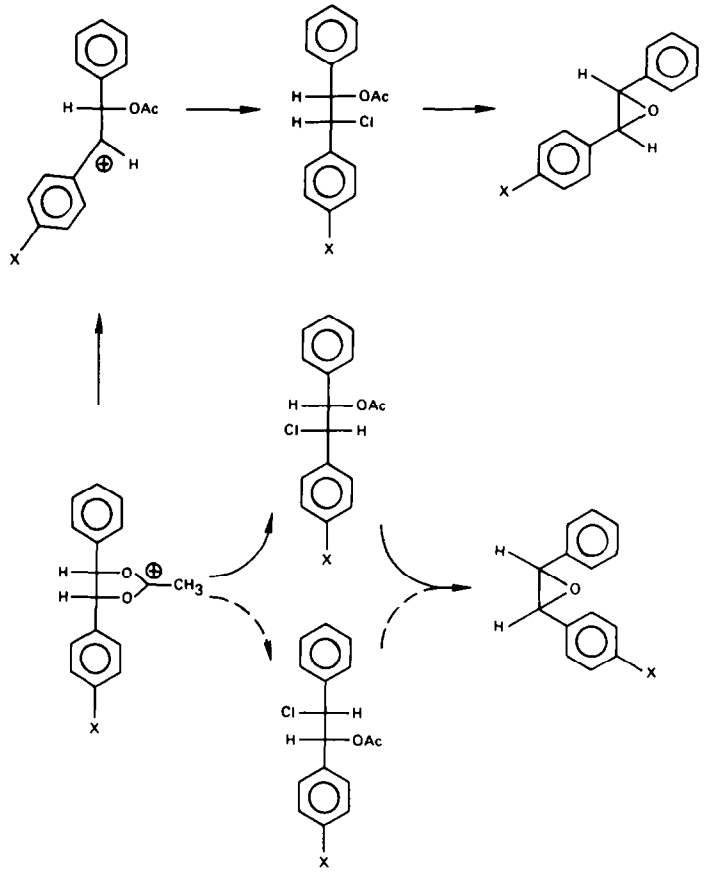
RESULTS AND DISCUSSION

Synthesis. In order to prepare optically pure substituted *cis* - 1,2 - diphenylethylene oxides by the Newman procedure,[13] optically pure *erythro* 1,2 - diphenylethane diols were required. These were conveniently obtained by the procedure outlined in Scheme 1. Reaction of either [*R*] - mandelonitrile[15] or [*S*] - mandelamide[16] with appropriately substituted (4-H,-Cl,-CH_3) phenylmagnesium bromides produced the substituted benzoins **1a–c**. Reduction of benzoins with metal hydrides is known to produce a mixture of *erythro* and *threo* diols.[17] The *erythro* diols **2a–c** (~70%) were readily separated from the *threo* isomers **3a–c** by fractional crystallization and were converted to the desired oxides **4a–c** essentially as described[13] for the preparation of optically pure styrene oxide.

Previous applications of the methoxydioxolane procedure[13] led to epoxides of the same configuration as the starting diols; i.e. *erythro* diols form *cis* substituted epoxides. However, in the present study, ~1:1 mixtures of *cis* (**4a–c**) and *trans* (**5a–c**) epoxides formed, each of which was highly optically active (Table 1, b and c series). The optical purity of **4b** was estimated as 92% via the use of an optically active shift reagent. The small loss in optical activity for **4b** probably occurred by a minor amount of racemization at the benzoin stage.[18] The optical purity of the *trans* epoxide **5b**, although unknown, is probably very high (Table 1). In addition, the absolute stereochemistry of 4'-chlorophenyl substituted carbon was inverted (see below). The optically pure *cis* epoxides are formed by cyclization of halohydrin acetates which were obtained by attack of chloride with inversion of configuration at either of the benzylic centers in the dioxolanes (Scheme 2). In order for the *trans* epoxides to be optically active, chloride must have preferentially attacked at only one of the benzylic centers of the dioxolane to form halohydrin acetates with retention of configuration. The absolute stereochemistry of the *trans* oxides **5b** and **c** (Table 1) requires that the precursor halohydrin acetates must have formed by a preferential attack of chloride with retention of configuration at the benzylic C atom with the chlorophenyl or methylphenyl substituent. If attack by chloride had occurred with retention of configuration with equal facility at the two benzylic C atoms, the resulting *trans* epoxide would have been racemic.



Scheme 1. Synthesis of optically active stilbene oxides, absolute stereochemistry is as indicated for the case in which X = Cl.



Scheme 1. Proposed mechanism for the formation of optically active substituted stilbene oxides.

Table 1. Physical properties of the optically active compounds

Compound			m.p.	(ref)	$[\alpha]_D$, $[\alpha]_{436}$[b]	c.d. $\theta(\lambda, nm)$	NMR[g]
benzoin	1a	[±][a]	132	(23)		-----	5.90
4-chlorobenzoin	1b	[2R]	91	(27)	-45, -132	-----	5.89
4-methylbenzoin	1c	[2S]	99	(15)	+83, +224	-----	6.08
erythro diphenylethane diol	2a	[meso]	134	(17)	-----	-----	4.82 (s)
erythro-1-(4′-chlorophenyl)-2-phenylethane diol	2b	[1S:2R]			-8, -17	+5,800 (225)	4.82 (s)
erythro-1-(4′-methylphenyl)-2-phenylethane diol	2c	[1R:2S]	106		-9, -18	-1,300 (227)	4.85 (s)
threo diphenylethane diol	3a	[1R:2R]	147	(17)	+92, +185	+37,680 (219)	4.60 (s)
threo-1-(4′-chlorophenyl)-2-phenylethane diol	3b	[1R:2R][c]	99		+125, +250	+50,300 (222)	4.60,4.61 (s)
threo-1-(4′-methylphenyl)-2-phenylethane diol	3c	[1R:2R]	97		+100, +222	+37,000 (220)	4.60 (s)
threo-1-(4′-nitrophenyl)-2-phenylethane diol	3d	[1R:2R][d]	112		+95, +255	+ 9,850 (215)	----
cis stilbene oxide	4a	[meso]	39	(14)	-----	------	4.36 (s)
cis-1-(4′-chlorophenyl)-2-phenylethylene oxide	4b	[1S:2R]	30		-35, -72	+12,110 (231)	4.30,4.32 (s)
cis-1-(4′-methylphenyl)-2-phenylethylene oxide	4c	[1R:2S]	28		+15, +29	-7,670 (227)	4.31 (s)
cis-1-(4′-nitrophenyl)-2-phenylethylene oxide	4d	[1S:2R][e]	59	(22)	+3.5, +52	-5,020 (255) -50,200 (222)	4.47
trans-stilbene oxide	5a	[R:R][f]	69	(27)	+360, ---	+80,000 (232)	3.83 (s)
trans-1-(4′-chlorophenyl)-2-phenylethylene oxide	5b	[1R;2R]	99	(27)	+350, +780	+59,130 (232)	3.85 (s)
trans-1-(4′-methylphenyl)-2-phenylethylene oxide	5c	[1S:2S]	61	(27)	-300, -580	-142,500 (232)	3.81 (s)
trans-1-(4′-nitrophenyl)-2-phenylethylene oxide	5d	[±]	126	(22)	-----	-----	3.87,4.0 (d,J=2)

[a] Both [*R*] **1a** ($[\alpha]_D = +119$) as well as [*R*] 4-methoxybenzoin ($[\alpha]_D = 111°$) have been described by J. Kenyon and R. L. Patel, *J. Chem. Soc.* (C), 435 (1965) and 97 (1966).

[b] Polarimetric measurements were made in ethanol at a concentration of 2–20 mg/ml except for **1b–c** where acetone was used.

[c] The optical purity was estimated by use of the NMR optical shift reagent *tris* - [3 - trifluoromethyl-hydroxymethylene) - d - camphorato] europium (III). The benzylic signal was split, and the signal of the [1*R*:2*S*] isomer moved downfield. By integration, the enantiomeric purity was found as 96%; 92% optical purity.

[d] The absorption spectrum of threo-**3d** is very different from that of **3a** making any ORD or cd assignment by analogy tenuous. A tentative assignment has been made on the basis of the preferred attacks by the enzyme at an (*S*) center.

[e] Additional treatment with epoxide hydrase caused no further change in specific rotation.

[f] Taken from Ref. 19.

[g] The position (ppm) of the benzylic hydrogens in $CDCl_3$ relative to internal TMS are presented.

Optical properties. Mason *et al.*[19] have correlated the sign of the CD bands of (+)-*trans*-stilbene oxide with its absolute stereochemistry by means of an exciton model in which the rotational strength of the long axis transition (~229 nm) is assumed to arise primarily from a Coulombic coupling of transitions of the two phenyl rings. The signs of the bands ~229 nm (Table 1) for the chloro and methyl substituted *trans*-stilbene oxides (**5b, c**) prepared in this study can be used to assign their absolute stereochemistry (Table 1). The results are consistent with inversion of configuration at the benzylic carbon bearing the substituted phenyl ring.

Although *cis*-stilbene oxide is optically inactive, the substituted isomers are optically active and are of unequivocal absolute stereochemistry by virtue of their synthesis. The CD spectra of the *cis* oxides (Table 1) shows that the ~229 nm transition is associated with two bands of opposite sign and equal magnitude. This suggests that the CD curves can be analyzed by an exiton model where the predominant effect arises from interaction of the two nearly degenerate aromatic bands (phenyl and *p*-Cl or *p*-Me phenyl). The role of the higher energy transition employed in Mason's[19] analysis of *trans* stilbene oxide can only be ascertained by detailed calculations in the case of the *cis*-stilbene oxides.

The synthetic *erythro* diols **2b, c** gave CD curves in the region of 220–227 nm, but the magnitudes of the effect was more than 10-fold smaller than the enzymatically formed *threo* isomers (see below). This difference in magnitude may be used to distinguish between optically pure *erythro* and *threo* isomers in this series, provided the substituents do not significantly alter the absorption spectra.

Enzymatic hydration. Optically active **4b, c** were incubated with liver microsomes from Sprague–Dawley rats. Examination of the NMR spectra of the resultant diols **3a–d** (Table 1), established that *trans* addition occurred yielding *threo* diols with either an [*R,R*] or [*S,S*] absolute stereochemistry. The CD bands in the 215–225 nm region for **3b, c** are similar to those of [*R,R*] **3a**[14] (Table 1). Since the chloro and methyl substituents do not significantly affect the sign, position, and magnitude of these bands[19] the absolute stereochemistry can be assigned as [*R,R*]. This establishes that the [*S*] carbon of the optically active epoxide **4b–c** was selectively attacked by water despite the fact that attack occurs at the C atom with the substituted phenyl ring ([*S,R*] **4b**) in one case and not in the other ([*R,S*] **4c**). Thus the stereochemistry of the C atom attacked and not the presence of the substituent controls the reaction. As anticipated from the above

observations, the specific optical activity of the diols **3b–d** was found to be independent of whether the substrates were optically active. Furthermore, when racemic oxide is incubated with epoxide hydrase, recovered oxide is optically active and has been enriched in the isomer that is hydrated at a slower rate (Table 1). Although the enzyme attacks exclusively at the [*S*] carbon atom of the epoxide, the reaction rate depends upon the substituent on the attached phenyl group.[20] Enantiomer [*S,R*] **4b** is enzymatically hydrated slower than the [*R,S*] **4b** isomer. Complete resolution of synthetic [*S,R*] **4b** was accomplished with epoxide hydrase, and the final rotation confirmed the initial estimate of 92% optical purity by NMR. Epoxide hydrase was employed as a reagent to effect the complete resolution of the epoxide **4d**. The observed absolute specificity for hydration at an [*S*] carbon atom by epoxide hydrase in 4'-substituted *cis* - 1,2 - diphenylethylene oxides as determined here by optical methods has been confirmed through the use of ^{18}O enriched water.[20]

EXPERIMENTAL

General. M.ps are uncorrected. NMR spectra were determined at 60 and 100 MHz. Specific rotations were determined with a Perkin–Elmer model 141 automatic polarimeter in a 1 ml, 10 cm cell, and CD spectra were measured with a Cary 60. [*R*]-(+)-Mandelonitrile was obtained in 95% yield on hydrolysis of amygdalin (Aldrich) by a modification of the method of Smith[15] where the desired product was continuously removed from the reaction medium by a layer of toluene. [*S*]-(+)-Mandelamide was prepared from [*S*]-(+)-mandelic acid.[16] A mixture of *cis*- and *trans* - 4 - nitrostilbene was obtained by reacting 4-nitrobenzaldehyde with (benzyl)-triphenylphosphonium chloride.[21] Oxidation of the mixture with *m*-chloroperoxybenzoic acid in methylene chloride for 4 hr followed by purification on thick layer chromatography (silica gel, benzene: petroleum ether (1:1)) provided the desired *cis* - 1 - (4' - nitrophenyl) - 2 - phenylethylene oxide.[22]

Enzymatic incubations. Microsomal pellets from the livers of phenobarbital treated male Sprague–Dawley rats (~150 g) were prepared as previously described.[23] The pellets were resuspended in Tris–HCl buffer (0.16 M, pH 9.0) containing 0.03% Tween 80 to a concentration of 2 mg/ml of protein. For each ml of incubation mixture, 1 mg of epoxide substrate in 50 μl CH_3CN was added and the mixture was incubated at 37° for various periods of time. Products were extracted into EtOAc (3×3 volume), dried (Na_2SO_4), concentrated, and separated by two successive thick layer chromatographies (silica gel; first, benzene: petroleum ether (1:1) and then $CHCl_3$: EtOAc (8:2)). The optical rotations and UV spectra were recorded in MeOH.

Enzymatic resolution of cis - 1 - (4 - *nitrophenyl*) - 2 - *phenylethylene oxide.* Racemic epoxide (30 mg) was incubated in 30 ml of microsomal suspension for 20 min. The epoxide and diol were separated, and the epoxide was recycled 5 times. The specific rotation was nearly constant after the third cycle ($[\alpha]_{436} = +52°$, 6 mg recovered after 5 cycles).

[*S*]-*p-Methylbenzoin.* A soln of 5 g of [*S*]-mandelamide in 60 ml THF was added to 200 mmole of tolylmagnesium bromide in 100 ml ether. The reaction was refluxed for 2 hr and hydrolysed by pouring into ice-water. The aqueous phase was extracted with ether before acidification with 10 ml conc H_2SO_4.[24] The benzoin slowly precipitated from the aqueous solution and was crystallized from 50% EtOH. An additional quantity was recovered from the ether phase using Girard T reagent.[25] The total yield was 70%.

[R]-p-*Chlorobenzoin.* A modification of the procedure of Smith,[15] in which Girard T reagent[25] replaced steam distillation in the purification provided the chlorobenzoin in 34% yield.

Hydrobenzoins. The above benzoins were reduced with $NaBH_4$ in EtOH.[17] Analysis of the crude mixture by NMR indicated a 7:1 ratio of *erythro* to *threo* diol. Two crystallization from 50% EtOH provided pure *erythro* diol.

Epoxides. Diols were heated for 2 hr at 100° with a 50% excess of trimethylorthoacetate in the presence of a trace of benzoic acid to form the corresponding methoxydioxolanes.[17] Excess trimethylorthoacetate was removed *in vacuo*. The crude mixtures were refluxed overnight in CH_2Cl_2 containing a 2-fold excess of trimethylchlorosilane to form chlorohydrin acetates in approximately 75% yield by NMR. Conversion to epoxides was effected by heating with 2 equivs of NaOH in isopropanol at 50° for 2 hr. The reactions were diluted with water and the products extracted into EtOAc which was dried and concentrated. Oxides were separated from recovered *erythro* diol by extraction of the oxides into a small volume of hexane. The NMR spectra of the crude oxides indicated a 1:1 mixture of *cis* and *trans* geometric isomers. The mixture was separated by thick layer chromatography (silica gel, benzene: petroleum ether (1:1)) to provide the *cis* (**4a–c**) and *trans* (**5a–c**) in overall yields of 35%, respectively.

REFERENCES

[1] J. W. Daly, D. M. Jerina and B. Witkop. *Experientia* **28**, 1129 (1972).

[2] J. W. Daly, *Handbook of Experimental Pharmacology* (Edited by B. B. Brodie and J. R. Gillette) Vol. XXVIII/2, p. 285. Springer, New York (1971).

[3] J. R. Holtzman, J. R. Gillette and G. W. A. Milne, *J. Biol. Chem.* **242**, 4368 (1967); *J. Am. Chem. Soc.* **89**, 6341 (1967).

[4] D. M. Jerina, H. Ziffer and J. W. Daly, *Ibid.* **92**, 1056 (1970).

[5] T. Watabe, K. Akamatsu and K. Kiyonaga, *Biochem. Biophys. Res. Commun.* **44**, 199 (1971).

[6] T. Watabe and K. Akamatsu, *Biochem. Biophys. Acta* **279**, 297 (1972); *Biochem. Pharmacol.* **23**, 1845 (1974).

[7] F. Oesch and J. W. Daly, *Biochem. Biophys. Acta* **222**, 692 (1971).

[8] F. Oesch, H. Thoenen and H. Fahrlaender, *Biochem. Pharmacol.* **23**, 1307 (1974).

[9] A. Y. H. Lu, D. Ryan, D. M. Jerina, J. W. Daly and W. Levin, *J. Biol. Chem.* **250**, 8283 (1975).

[10] D. M. Jerina and J. W. Daly, *Science* **185**, 573 (1974).

[11] P. Sims and P. L. Grover, *Adv. Cancer Res.* **20**, 165 (1974).

[12] D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman Nirenberg and S. Udenfriend, *Biochemistry* **9**, 147 (1970).

[13] M. S. Newman and C. H. Chen, *J. Am. Chem. Soc.* **94**, 2149 (1972), **95**, 278 (1973); M. S. Newman and D. R. Olson, *J. Org. Chem.* **38**, 4203 (1973). See also C. U. Pittman, S. P. McManus and J. W. Larsen, *Chem. Revs* **72**, 357 (1972).

[14] G. Berti, F. Bottari and B. Macchia, *Il Farmaco*-Ed. Sc. XV, 377 (1960).

[15] I. A. Smith, *Dtsch. Chem. Ges.* **62**, 429 (1931) and A. Weissberger, E. Strassen, H. Mainz and W. Schwarze, *J. Liebigs Ann.* **478**, 112 (1930).

[16] L. F. Audrieth and M. Sveda, *Org. Synthesis*, Coll. Vol. 3, p. 536, Wiley, New York (1955).

[17] H. O. House, *Modern Synthetic Reactions*, 2nd Edn, p. 57. Benjamin, Menlo Park (1972).

[18] R. Roger and R. Wood, *J. Chem. Soc.* 811 (1954).

[19] I. Moretti and G. Torre, *Tetrahedron Letters* 2717 (1969); G. Gottarelli, S. F. Mason and G. Torre, *J. Chem. Soc.* (B) 1349 (1970); P. Crabbe, *ORD and CD in Chemistry and Biochemistry, An Introduction* 1. Academic Press, New York (1972).

[20] P. Dansette, V. Makedonska and D. M. Jerina, to be published.

[21] R. Ketcham, D. Jambotkar and L. Martinelli, *J. Org. Chem.* **27**, 4666 (1962); and A. W. Johnson and V. L. Kyllingstad, *Ibid.* **31**, 334 (1966).

[22] E. Bergman and J. Hervey, *Dtsch. Chem. Ges. Ber.* **62**, 893 (1929).

[23] H. Slenader, D. M. Jerina and J. W. Daly, *Arch. Biochem. Biophys.* **168**, 309 (1975).

[24] A. McKenzie, G. Martin and H. G. Rule, *J. Chem. Soc.* **105**, 1583 (1914) and A. McKenzie and H. Wren, *Ibid.* **93**, 309 (1908).

[25] L. F. Fieser and M. Fieser, *Reagents in Organic Chemistry*, Vol. I. p. 410. Wiley, New York (1967).

[26] S. S. Jenkins, *J. Am. Chem. Soc.* **56**, 682 (1934).

[27] A. Feldstein and C. A. Vanderverf, *Ibid.* **76**, 1626 (1954).

[28] C. Cecarelli, G. Berti, G. Lippi and B. Macchia, *Org. Magn. Resonance* **2**, 379 (1970); I. Moretti, F. Taddel, G. Torre and N. Spassky, *J. Chem. Soc.* Chem. Commun. 25 (1973).