BULLETIN
OF THE KOREAN CHEMICAL SOCIETY

VOLUME 10, NUMBER 4
AUGUST 20, 1989

BKCS 10(4) 331-410
ISSN 0523-2964

Peptide Synthesis with Polymer Bound Active Ester. I. Rapid Synthesis of Peptides Using Polymer Bound 1-Phenyl-3-methyl-4-oximinopyrazole

Ki-Wha Lee and Yoon-Sik Lee*

Department of Chemical Technology, College of Engineering, Seoul National University, Seoul 151-742

Received February 3, 1989

Polymer bound 1-phenyl-3-methyl-4-oximinopyrazoles were prepared through a series of chemical modifications of Merrifield's resin (chloromethylpolystyrene-1% DVB-copolymer). Several polymer active esters of N-blocked amino acids were prepared from the polymer bound 1-phenyl-3-methyl-4-oximinopyrazoles. Polymer bound active esters were found to be highly reactive in N-acylation reaction. The resins were tested for the preparation of several dipeptides. The peptides were obtained in high yields within 10 minutes and the progress of the reactions could be easily followed up by the color change of the resin. The resulting peptides were characterized by NMR and other physical methods.

Introduction

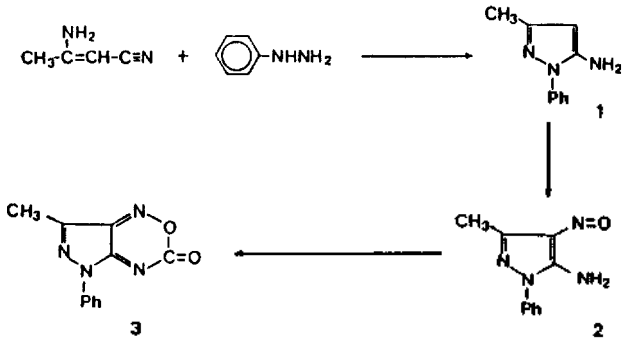
Nowadays peptide synthesis using polymers by Merrifield's method[1] has been a routine procedure. Being different from the typical Merrifield's methods, polymer bound active ester method in which polymer support acts as an acyl carrier are suitable for the synthesis of short peptides and semisynthetic penicillins or cephalosporins. Polymer bound active esters are easily separated from low molecular weight reactants and by-products by simple filtration. They can be used in excess to affect fast and quantitative acylations, and are usually recyclable.

Various types of active esters have been successfully used in solid phase as well as in solution phase for the stepwise synthesis of peptides with predetermined sequences.[2] But solid phase peptide synthesis using active esters of oxime type group has been rarely reported. Even though there are various kinds of oxime type group reported in peptide synthesis[3,4], there has been less attention to use them as acyl carriers in solid phase peptide synthesis. Among them, 4-nitroso-5-aminopyrazoles which contain an oxime group in tautomeric form drew much of our attention. With two moles of acylating agents, they can be converted to 4-acyloximino-5-acylimino-2-pyrazolines, which react fast with one mole of amino acid esters yielding peptide compounds and 4-nitroso-5-acylaminopyrazoles. The N-acylation reaction was reported to be very fast and could be easily followed by the color change of the reaction solution.[4] We now wish to report on the synthesis and the properties of polymer bound 1-phenyl-3-methyl-4-oximinopyrazoles[5] which contain succinoyl or ϵ-aminocaproyl spacer arms.

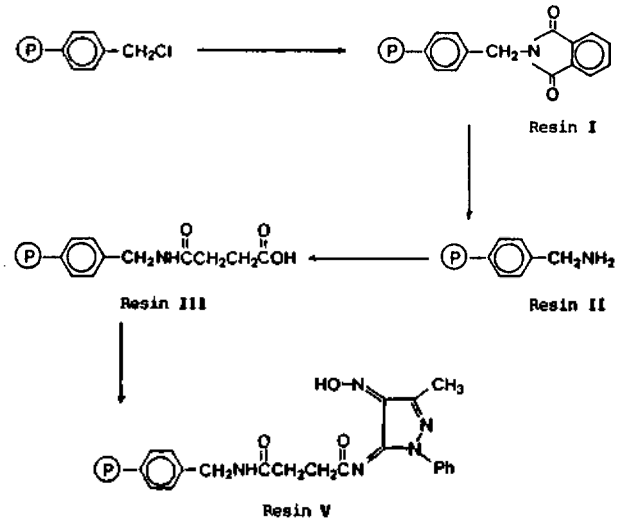
Results and Discussions

1-Phenyl-3-methyl-5-aminopyrazole(**1**) has been synthesized by Mohr[6], Taylor[7] and Guarneri[8]. But their synthetic methods have shown some difficulties requiring several steps starting from cyanoacetone. However, we obtained it by simple refluxing β-aminocrotonitrile and phenylhydrazine in ethanol until ammonia evolution had ceased. It took rather a long time (about 40 hours) but, **1** was easily obtained in good yield. Nitrosation of **1** in aqueous acid medium gave 1-phenyl-3-methyl-4-nitroso-5-aminopyrazole(**2**) as red solids. **2** was reacted with ethyl chloroformate in the presence of triethylamine(TEA). After removing TEA·HCl salt, the resulting 4-nitroso-5-(ethoxycarbonylamino)pyrazole was refluxed in benzene to afford the ring closed product, 5-phenyl-7-methylpyrazolo[4,3-c][1,2,4]oxadiazin-3-one (**3**). **3** was very reactive to amino group but rather stable to hydroxy group.

A polymer support to condense with **2** or **3** was prepared through a series of chemical modifications of Merrifield's resin (chloromethylpolystyrene-1% DVB-copolymer, 1.0 meq. Cl^-/g). According to Sparrow's procedure[9], the displa-



Scheme 1



Scheme 2

cement of chloride from Merrifield's resin with potassium phthalimide in dimethylformamide proceeded smoothly to give Resin I. Treatment of Resin I with hydrazine hydrate in refluxing ethanol overnight gave Resin II. Free amino group determination by Gisin's method[10] indicated approximately 1 meq. of amino group per unit gram of the resin. It was well agreed with the result of elemental analysis of nitrogen. Resin II was condensed with succinic anhydride to give Resin III. Resin III was coupled with 2 by using dicyclohexylcarbodiimide(DCC) and 1-hydroxybenzotriazole(HOBt) in tetrahydrofuran. The color of the resulting Resin V was changed from white to green. Based on the degree of substitution calculated from elemental analysis of nitrogen, several N-protected amino acids were anchored to Resin V by DCC method. The IR spectra of the resulting Boc(or Z)-AA-Resin V displayed new carbonyl band at 1800 cm^{-1} and the color of the resin beads turned red from green. Boc(or Z)-AA-Resin V was stable in water or alcohols for several hours at room temperature. The degree of substitution of N-protected amino acid was determined by reaction with excess of benzylamine followed by back titration with perchloric acid.[2d] In order to demonstrate the coupling efficiency of the polymer bound active ester, Boc(or Z)-AA-Resin V, in peptide synthesis several dipeptides were prepared. The coupling reactions with amino acid esters were normally finished within 10 minutes at room temperature in good yields and could be easily followed by the color change of the resin. Table 1 summarizes the experimental results. Run 1-7 and

Table 1. Dipeptide Derivatives Prepared from Boc(or Z)-Phe-Resin V[a]

Run	Peptide	Active ester/amino acid ester (mol/mol)	Rxn. time (min)	Yield (%)
1	Boc-Phe-Gly-OMTP	0.80:1	30	98
2	Boc-Phe-Gly-OMTP	0.80:1	20	76
3	Boc-Phe-Gly-OMTP	0.94:1	20	79
4	Boc-Phe-Leu-OBzl	0.95:1	10	75
5	Boc-Phe-Leu-OBzl	0.77:1	10	88
6	Boc-Phe-Leu-OBzl	0.74:1	10	62
7	Boc-Phe-Leu-OBzl	1.10:1	10	52
8	Z-Phe-Gly-OBzl	1.35:1	10	58
9	Z-Phe-Leu-OMe	1.33:1	10	70
10	Z-Phe-Phe-OMe	1.45:1	10	70
11	Z-Phe-Gly-OBzl	0.97:1	10	65

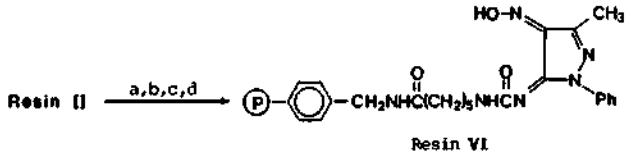
[a]The same resins were used in Run 1 to 7 and 8 to 11 respectively.

Table 2. Dipeptide Derivatives Prepared from Boc(or Z)-AA-Resin VI[a]

Run	Peptide	Active ester/amino acid ester (mol/mol)	Rxn. time (min)	Yield (%)
1	Boc-Gly-Gly-OBzl	1.43:1	10	76
2	Z-Phe-Gly-OBzl	1.50:1	10	87
3	Z-Phe-Leu-OMe	1.33:1	10	95
4	Z-Phe-Leu-OMe	1.33:1	7	77
5	Z-Phe-Phe-OMe	1.52:1	10	90

[a]The same resin was used from Run 2 to 5.

Run 8-11 were the results of the same resins which were repeatedly acylated 7 or 4 times with the previous amino acid derivatives after each coupling reaction. These results showed that Resin V could be reused several times without significant loss of reactivity. But reusability of Resin V was not so satisfactory. Coupling yield of Resin III with 2 was rather low (ca. 70%) and the yields of peptide products seemed decreased as the resins were repeatedly used. Moreover, the amide bond between the Resin III and 2 was found to be slightly reactive to nucleophile when the resin was exposed several hours to free amino group during coupling reactions. TLC of the reaction mixture conformed the loss of some pyrazole groups during the repeated coupling reactions. When Resin V was exposed to acyl chloride in reaction mixture, the color of the resin beads became yellow, and all the signs as reusable active ester seemd vanished. To compensate for these drawbacks the anchoring method of pyrazole group to the resin was modified.



Scheme 3

Table 3. Physical Data of Dipeptide Derivatives[a]

Peptides	R_f (TLC)[b]	mp(°C)	$[\alpha]_D$(c, solvent, °C)	*Lit.*	
				mp(°C)	$[\alpha]_D$(c, solvent, °C)
Boc-Gly-Gly-OBzl	0.42(A)	79–81		77– 79[11]	
Boc-Phe-Gly-OMTP	0.81(A)	150–151	−12.2(1.0, $CHCl_3$, 25)	148–149[17]	−7.0(1.0, AcOH, 20)
Boc-Phe-Leu-OBzl	0.80(A)	81–82	−26.7(1.0, MeOH, 25)	82– 84[18]	−23.8(1.0, MeOH, 25)
Z-Phe-Gly-OBzl	0.71(A)	130–131	−25.2(0.4, MeOH, 25)	130–131[12]	
Z-Phe-Leu-OMe	0.71(A)	101–103	−25.5(3.1, MeOH, 25)	110–111[13]	−24.7(3.1, MeOH, 22)
Z-Phe-Phe-OMe	0.68(A)	139–140	−17.4(0.26, MeOH, 25)	138–140[14]	−20.0(0.26, MeOH, 25)

[a] ^{1}H NMR data and elemental analysis data of all the peptide derivatives were satisfactory. [b] Solvent systems are described in experimental section.

Table 4. Comparision of Polymer Bound 1-Phenyl-3-methyl-4-oximinopyrazole and Polymer Bound 1-Hydroxybenzotriazole[2a,b]

	Polymer bound 1-Phenyl-3-methyl-4-oximinopyrazole	Polymer bound HOBt
N-acylation reactivity	with 10 min.	10–30 min.
Reactivity to -OH group	stable	slightly reactive
Monitoring method	color change	TLC

Boc-ε-aminocaproic acid (Boc-ACA) was coupled to Resin II by using DCC in methylene chloride. After the coupling reaction of Boc-ACA was repeated, it was treated with 25% trifluoroacetic acid and neutralized with 10% DIEA to afford Resin IV. Another polymer bound 1-phenyl-3-methyl-4-oximinopyrazole was made from ring opening condensation of 3 with Resin IV. The color of the resulting Resin VI was also changed immediately from white to green. Resin VI was tested as an acyl carrier in the same manner as done with Resin V. Table 2 summarizes the experimental results. Yields were more increased, and the same reactivity was observed. Moreover, Resin VI seemed superior to Resin V in stability toward amino group. The results from Run 2 to 5 indicates that after the coupling reactions were finished, Resin VI can be reanchored several times with the acyl group of the previous amino acid derivatives without any loss of functional groups. Unlikely to Resin V which anchored the pyrazole group through amide bond, the pyrazole groups in Resin VI was connected to the resin through urea bond which showed much more stability toward aminolysis. The physical properties of the isolated peptide derivatives are listed in Table 3.

Some characteristic properties of polymer bound 1-phenyl-3-methyl-4-oximinopyrazole and polymer bound 1-hydroxybenzotriazole (HOBt)[2a,b] are summarized in Table 4. So far the polymer bound HOBt was known to be one of the most reactive active ester resins ever reported. The polymer bound 1-phenyl-3-methyl-4-oximinopyrazole (Resin VI) which has ε-aminocaproyl spacer group showed almost the same reactivity as polymer bound HOBt and good stability. Moreover, the coupling reaction could be easily followed by the color change of the resin.

Experimental

All the chemicals used were of reagent grade and purified prior to use, if necessary, by the methods reported in the literature. Melting points were measured on a Yanaco MP-S5 and are not corrected. IR spectra were obtained by Jasco DS-710 IR spectrophotometer and ^{1}H-NMR spectra were recorded on a Jeol JNM-MH-100 NMR spectrometer. The internal standard was TMS. Optical rotations were measured by Jasco DIP-360 polarimeter and elemental analyses were performed with Yanaco MT-2 CHN coder. Analytical thin layer chromatography was performed on silica gel plate (0.25 mm, 60F-254, E. Merck) with the following solvent systems; A, chloroform/methanol (15:1); B, chloroform/methanol (25:1).

1-Phenyl-3-methyl-5-aminopyrazole(1). A solution of β-aminocrotonitrile (25 g, 295.3 mmol) and phenylhydrazine (35 m*l*, 355.7 mmol) was refluxed in ethanol until ammonia evolution had ceased (about 40 hours). Evaporation of the reaction mixture under reduced pressure yielded a reddish brown residue which was crystallized from benzene to give white needles, 36.8 g(72%), mp 110–111 °C (lit.[6] 115–116 °C); Rf 0.61(A); NMR($CDCl_3$) δ 2.25(s, 3H), 3.85 (broad, 2H), 5.4(s, 1H), 7.2–7.6 (m, 5H); Anal. Calcd. for $C_{10}H_{11}N_3$ (173.22): C, 69.34; H, 6.40; N, 24.26%. Found: C, 69.64; H, 6.51; N, 23.84%.

1-Phenyl-3-methyl-4-nitroso-5-aminopyrazole(2). To a solution of 1-phenyl-3-methyl-5-aminopyrazole(**1**) (15 g, 86.6 mmol) in 0.5 N aqueous HCl (200 m*l*), maintained at 0–5 °C, was added with stirring a solution of sodium nitrite (7.77 g, 112.6 mmol) in water (30 m*l*) in 50 minutes. Stirring and chilling were continued for 40 minutes after all the sodium nitrite solution had been added. Precipitated reddish-brown solids were then collected by filtration and washed with ice-water. Recrystallization from $CHCl_3$ yielded red prisms, 12.84 g (73%), mp 201–202 °C (lit.[15] 199–200 °C); Rf 0.73(A); NMR ($CDCl_3$) δ 2.85 (s, 3H), 7.15 (broad, 2H), 7.4–8.0 (m, 5H); Anal. Calcd. for $C_{10}H_{10}N_4O$ (202.22): C, 59.40; H, 4.98; N, 27.71%. Found: C, 59.73; H, 4.82; N, 27.49%.

5-Phenyl-7-methyl[4,3-c][1,2,4]oxadiazin-3-one(3). Triethylamine (1.39 m*l*, 9.96 mmol) and ethyl chloroformate (0.95 m*l*, 9.97 mmol) were added to a stirred solution of 1-phenyl-3-methyl-4-nitroso-5-aminopyrazole(**2**) (2 g, 9.89 mmol) in methylene chloride (100 m*l*) at 0–5 °C. The solution turned immediately red and became green after 2 hours. After the solution was evaporated to an oily residue, ethyl ether was added to give solid. It was filtered and the solution was evaporated again. The remaining oily residue was refluxed in benzene (200 m*l*) for 5 hours. The solution

became reddish-brown. Evaporation of the reaction mixture under reduced pressure yielded red solid, which was crystallized from EtOAc-pet. ether. Yield 1.77 g (78%); mp 148–150 °C; Rf 0.63 (B); NMR ($CDCl_3$) δ 2.65 (s, 3H), 7.4–8.4 (m, 5H); Anal. Calcd. for $C_{11}H_8N_4O_2$ (228.21): C, 57.89; H, 3.53; N, 24.55%. Found: C, 58.15; H, 3.53; N, 24.04%.

Phthalimidomethylpolystyrene (Resin I). Chloromethylpolystyrene-1% DVB-copolymer (Aldrich, 10 g, 1 meq. Cl^-/g) was suspended in freshly distilled DMF and potassium phthalimide (1.86 g, 10 mmol) was added. The mixture was stirred at 50 °C for 18 hours, after which the resin was washed three times with DMF, methanol, water, methanol and dried in vacuo overnight. IR(KBr) 1710, 1775 cm^{-1}; Chloride anal.[9], 0.03 meq./g resin.

Aminomethylpolystyrene (Resin II). Resin I (8.2 g) was treated for 16 hours with hydrazine hydrate (1 m*l*) in refluxing ethanol. The resin was filtered from hot ethanol and washed three times each with hot ethanol, 5% aqueous KOH, water, ethanol and dried in vacuo overnight. Amino group anal.[10], 1.01 meq/g resin.

3-(Polystyrylmethylenecarbamoyl)propionic acid (Resin III). Resin II (6 g) was stirred with succinic anhydride (6 g, 60 mmol) in DMF at 50 °C until ninhydrin test[16] showed negative. The resin was filtered and washed with DMF, water, methanol, methylene chloride and dried in vacuo overnight. N, 1.25% (0.89 meq./g resin).

6-Aminohexanamidomethylpolystyrene (Resin IV). Resin II (3 g) was transferred to a solid phase reaction vessel[16] and washed with methylene chloride. Boc-ε-aminocaproic acid (1.04 g, 4.5 mmol) in methylene chloride (20 m*l*) and DCC (1 g, 4.84 mmol) in methylene chloride (5 m*l*) was then added successively and shaken for 12 hours. The resin was filtered and washed three times each with methanol/methylene chloride (1:2) and methylene chloride. The coupling reaction was repeated as the above and the resin was washed in the same manner. The Boc group was removed by treating with 25% trifluoroacetic acid/methylene chloride (30 min) and neutralized with 10% DIEA/methylene chloride. Then the resin was washed with methylene chloride and dried in vacuo overnight. Amino group anal.[10], 0.84 meq./g resin.

1-Phenyl-3-methyl-4-oximino-5-[N-3-(polystyrylmethylenecarbamoyl)propionyl]iminopyrazole (Resin V). Resin III (2 g) was treated with HOBt (0.34 g, 2.52 mmol) and DCC(0.48 g, 2.33 mmol) in tetrahydrofuran for 30 minutes. **2** (1.17 g, 5.79 mmol) was then added and shaken for 12 hours. The resin was filtered and washed three times with tetrahydrofuran. The coupling reaction was repeated with **2** (0.99 g, 4.9 mmol), HOBt (0.26 g, 1.92 mmol) and DCC (0.46 g, 2.23 mmol). The resin was then filtered and washed three times each with tetrahydrofuran, isopropyl alcohol/methylene chloride (1:1), DMF methylene chloride, and dried in vacuo. IR (KBr) 1660, 1710 cm^{-1}; N, 4.27% (0.54 meq./g resin).

1-Phenyl-3-methyl-4-oximino-5-N-(polystyrylmethylenecarbamoyl pentamethylenecarbamoyl)-iminopyrazole (Resin VI). Resin IV (3.15 g) was treated in methylene chloride with 5-phenyl-7-methyl[4,3-c][1,2,4]-oxadiazin-3-one (**3**) (0.7 g, 3.07 mmol) for 75 minutes. The color of the beads became immediately green from white. The resin was washed three times each with methanol and methylene chloride. IR (KBr) 1660, 1710 cm^{-1}; N, 5.77% (0.67 meq./g resin).

Typical Procedure for the Preparation of Active Esters. Resin V (or VI) was treated with 2 equivalents of Boc (or Z)-amino acid and 2.1 equiv. of DCC in methylene chloride for 5 hours. The color of the beads turned red from green. The resin was washed three times each with methylene chloride, methanol/methylene chloride (1:1), methylene chloride and dried in vacuo. IR (KBr) 1660, 1710, 1800 cm^{-1}; Amino group anal.[2d], 0.36 mmol/g (Boc-Phe-Resin V); 0.40 mmol/g (Z-Phe-Resin V); 0.44 mmol/g (Boc-Gly-Resin VI); 0.50 mmol/g (Z-Phe-Resin VI).

Typical Procedure for Peptide Synthesis (Z-Phe-Leu-OMe). Z-Phe-Resin VI (2.4 g, 1.2 mmol) was swelled with methylene chloride (25 m*l*) in the reaction vessel. TsOH·Leu-OMe (285 mg, 0.9 mmol) was suspended in methylene chloride (5 m*l*) and neutralized with DIEA. The resulting solution was poured into the reaction vessel and the reaction mixture was shaked for 10 minutes at room temperature. The color of the beads became green from red. The resin was filtered and washed with methylene chloride. The filtrate and the wash were combined. The combined solvent was washed with 10% citric acid (3×10 m*l*), water (2×10 m*l*) and dried with Na_2SO_4. Evaporation of the solvent gave an oily residue which was crystallized in EtOAc-pet. ether to give white needle crystals, 365 mg (95%, based on the amount of TsOH·Leu-OMe).

Acknowledgement. We thank Korea Research Institute of Chemical Technology for financial support.

References

1. (a) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); (b) G. Barany and R. B. Merrifield, In "The Peptides" (E. Gross and J. Meienhofer Ed.), Vol. 2, pp. 1-284, Academic Press, New York (1980).
2. (a) M. Fridkin, A. Patchornik, and E. Katchalski, *J. Am. Chem. Soc.*, **88**, 3164 (1966); (b) M. Fridkin, A. Patchornik, and E. Katchalski, *ibid.*, **90**, 2953 (1968); (c) M. Fridkin, A. Patchornik, and E. Katchalski, *Bochemistry*, **11**, 466 (1972); (d) R. Kalir, M. Fridkin, and A. Patchornik, *Eur. J. Biochem.*, **42**, 151 (1974); (e) R. Kalir, A. Warshawsky, M. Fridkin, and A. Patchornik, *ibid.*, **59**, 55 (1975).
3. (a) G. Losse, A. Barth, and K. Schatz, *Liebigs Ann. Chem.*, **677**, 185 (1964); (b) G. Losse, K.-H. Hoffman, and G. Hetzer, *ibid.*, **684**, 236 (1965).
4. M. Guarneri, P. Giori, and C. A. Benassi, *Tetrahedron Lett.*, 665 (1971).
5. Guarneri *et al.* have first exploited 4-nitroso-5-aminopyrazole by attaching it to a polystyrene resin through urethane bond. (see, p.338 of Ref. 1(b)).
6. E. Mohr, *J. Prakt. Chem.*, [2], **79**, 16 (1909).
7. E. C. Taylor and K. S. Hartke, *J. Am. Chem. Soc.*, **81**, 2456 (1959).
8. M. Guarneri, R. Ferroni, and F. Fiorini, *Gazz. Chim. Ital.*, **98**, 569 (1968).
9. J. T. Sparrow, *J. Org. Chem.*, **41**, 1350 (1976).
10. B. F. Gisin, *Anal. Chim. Acta.*, 58 (1972).
11. J. Goodacre, L. Jeffries, J. H. C. Nayler, R. J. Ponsford, and I. Stirlings, *J. Med. Chem.*, **20**, 1445 (1977).
12. D. Theodoropoulos and J. Gazopoulos, *J. Org. Chem.*,

27, 2091 (1962).
13. S. Yamada and Y. Takeucki, *Tetrahedron Lett.*, 3595 (1971).
14. M. A. Tilak and J. A. Hoffmann, *J. Org. Chem.*, **42**, 2098 (1977).
15. E. Mohr, *J. Prakt. Chem.*, [2], **79**, 26 (1909).
16. J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", 2nd Ed., Pierce Chemical Company, Rockford, Illinois (1984).
17. D.-H. Park, J.-K. Jung, and Y.-S. Lee, *Bull. Korean Chem. Soc.*, **9**(6), 394 (1988).
18. P. B. W. T. Kortenaar, W. W. Wilkerson, N. T. Boggs, III, D. A. Modar, K. A. Koehler, and R. G. Hiskey, *Int. J. Pep. Protein Res.*, **16**, 440 (1980).

Crystal Structure of Cholesteryl Methyl Ether

Mi Kyung Yun, Young Ja Park*, Whanchul Shin†, and B. M. Craven‡

Department of Chemistry, Sook Myung Women's University, Seoul 140-742
†Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742
‡Department of Crystallography, University of Pittsburgh, Pittsburgh, Pa., 15260, U.S.A.
Received February 8, 1989

Cholesteryl methyl ether($CH_3OC_{27}H_{45}$) crystallizes in the monoclinic space group $P2_1$ with a = 11.740 (8), b = 7.576 (5), c = 15.492 (10)Å, β = 110.39 (5)°, Z = 2, Dc = 1.03 g/cm^3 and Do = 0.96 g/cm^3. The intensities were collected on a Nonius CAD-4 diffractometer with Nb-filtered Mo-K_α radiation. The structure was solved by direct methods and refined by full matrix least-squares methods. The final R factor was 0.085 for 1479 observed reflections. Compared with other cholesterol derivatives, the cholesteryl ring and tail region of the molecule are normal. The molecular long axes are parallel to the [101] axis and molecules are packed together in a similar way to those in cholesteryl chloride and bromide.

Introduction

Cholesterol is the major sterol in animal tissues. Cholesterol and its esters with fatty acids are important components of plasma lipoproteins and outer cell membrane. Cholesterol derivatives are important constituents of pathological conditions such as atherosclerosis, the formation of thick deposits of cholesterol and its esters on the inner surfaces of blood vessels, and the factors that determine their mode of crystallization and packing may be useful in understanding their deposition in arteries. In addition, the allowable tail conformations will help define permissible interactions with membrane constituents such as phospholipids.[1,2]

Although the conformation of the nucleus of cholesterol has been well established by a number of structure determinations, there are still several reasons for solving the structures of cholesterol derivatives. Though the crystal structures of many cholesterol ester derivatives[3-17] were solved, the structure of a cholesterol ether derivative has not yet been reported.

From consideration of the crystal data of the cholesteryl methyl ether, the molecules are showing a tendency to pack together in a different way to those found in other cholesterol esters. In order to investigate the structural characteristics, bond distances, bond angles, torsion angles of rings and side chain, packing mode and intermolecular interactions, the structure analysis of the cholesteryl methyl ether has been undertaken.

Experimental

Cholesteryl methyl ether was obtained from Sigma Chemical Company. Colorless prismatic crystals having their longest dimension in the b-axis direction were grown by slow evaporation of a saturated aceton solution. Oscillation and Weissenberg photographs, which showed systematic absent reflections for $0k0$ when k is odd, indicated that crystals are monoclinic with space group $P2_1$. The X-ray intensity data were measured on the Nonius CAD-4 diffractometer with Nb-filtered Mo-K_α radiation (λ = 0.7107Å). A cystal having dimensions of 0.3 mm × 0.8 mm × 0.5 mm was used in the experiment. The approximate unit cell parameters which were obtained from low angle reflections agreed with those reported earlier by Bernal, Crowfoot and Frankuchen[3]; a = 1.72, b = 7.58, c = 22.40 Å, β = 139.7°. However, this unit cell was so oblique that a new unit cell was chosen. The unit cell parameters were determined by a least-squares fit of 2θ angles for 33 reflections (14° < θ < 18°).

Detailed crystal data are as follows. $C_{28}H_{48}O$; mol. wt. 400.7; space group, monoclic, $P2_1$; z = 2; a = 11.740 (8), b = 7.576 (5), c = 15.492 (10) Å, β = 110.39 (5)°, V = 1291.47 Å^3; μ(Mo-K_α) = 0.64 cm^{-1}; D_c = 1.03 g/cm^3, D_m = 0.96 g/cm^3 measured by floatation method in a mixture of water and isopropyl alcohol. The intensity data were collected with Nb-filtered Mo-K_α radiation using the $\theta/2\theta$ scan techniques. The intensities of three reflections ($\bar{1}04$, 500, 020) were monitored every 30 reflections throughout the data collection and showed no systematic variations. The 4013 independent reflections were measured, range of hkl: $-15 \le h \le 15, 0 \le k \le 10$ and $0 \le l \le 20$, within $2° \le 2\theta \le 60°$.