Scheme II

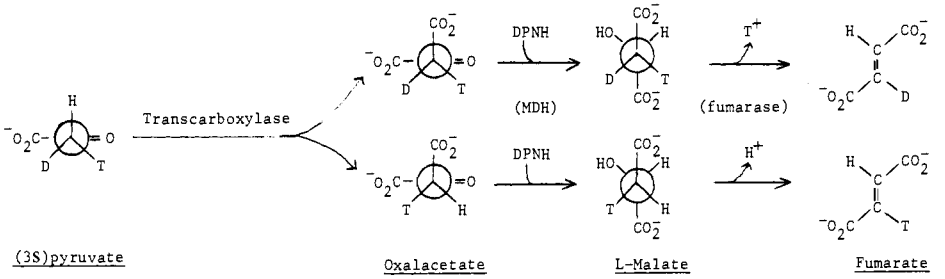


Table I. Stereospecificity of Ketonization by Pyruvate Kinase[a]

expt	precursor of L-malate-*3-t*	fractional release of tritium from L-malate by fumarase: individual	average[c]
1	pyruvate-*3-t*[b]	0.496	0.498 ±
		0.500	0.009
2	(*E*)-PEP-*3-t* + acid	0.484	0.491 ±
	phosphatase	0.479	0.010
		0.495	
		0.497	
		0.497	
3	(*E*)-PEP-*3-t* + ADP +	0.626	0.630 ±
	pyruvate kinase	0.628	0.006
		0.636	
		0.629	
4	(*E*)-PEP-*3-t* + pyruvate	0.618	0.616 ±
	kinase +	0.612	0.006
	acid phosphatase	0.616	

[a] Incubations in 0.5 mL of D_2O at 15 °C contained sodium maleate buffer (50 mM, pD 6.4), $MgCl_2$ (1 mM), PEP (1 mM), and the additions noted. Acid phosphatase of potatoes (2.5 mg) was from Sigma Co. It caused hydrolysis of PEP at ~0.7 μmol/min under the conditions used. Rabbit muscle pyruvate kinase, from Boehringer (0.2 mg ≃ 40 U), was added with $(NH_4)_2SO_4$ (~10 μmol). ADP was present at 1 mM in the incubation lacking phosphatase. [b] This malate was made from achiral pyruvate-*3-t*, transcarboxylase, and MDH. It was purified through silicic acid column. [c] 95% confidence limit.

drawn with the assumption that pyruvate kinase ketonizes enol pyruvate with the same stereospecificity as has been shown with PEP and ADP.[15] When pyruvate production was complete, as judged by assay with lactate dehydrogenase on control samples, $HClO_4$ was added to denature the enzymes and $KHCO_3$ added to neutralize. The precipitate was removed by centrifugation. Pyruvate in the supernatant was converted to malate by action of transcarboxylase with methylmalonyl CoA and malate dehydrogenase (MDH) and reduced diphosphopyridine nucleotide (DPNH), as observed at 340 nm. The L-malate was isolated by ion-exchange chromatography on Dowex-1-Cl^-. Its radiochemical purity was shown to be >96% by a modified procedure[15] in which malate was treated with fumarase followed by MDH plus the 3-acetylpyridine analogue of diphosphopyridine nucleotide and the radioactivity shown to be in water.

The tritium present at the *pro R* position of C-3 of the malate was determined by treatment with fumarase and measurement of the fraction of tritium released to water.[15-17] These steps for determining the chirality of the pyruvate are shown in Scheme II.

As seen in Table I, experiment 1, fumarase labilizes 50% of the tritium of L-malate-*3-t* generated from achiral pyruvate-*3-t* as expected.[6] Experiment 2 shows that pyruvate formed from enolpyruvate nonenzymatically is a racemic mixture. Kinetic studies have also shown that the phosphatase does not catalyze the ketonization.[14] When the (*E*)-PEP-*3-t* was converted to pyruvate by pyruvate kinase and ADP, without acid phosphatase as in experiment 3, the product was (3*S*)-pyruvate in agreement with previous studies.[15] The isotope effect exhibited here by transcarboxylase, which is shown by the ratio of (3*R*)- to (3*S*)-malate-*3-t* formed agreed with the intramolecular discrimination reported.[18] When ADP is omitted there was no production of pyruvate unless acid phosphatase is added. In this case, experiment 4, the pyruvate was formed with the same stereochemistry observed in the overall reaction. This result indicates that pyruvate kinase catalyzes the formation of pyruvate from the intermediate generated by action of phosphatases on PEP by a ketonization mechanism by proton approach to C-3 from the *si* face of enolpyruvate. At the concentration of pyruvate kinase present almost all of the ketonization was enzymatic. This result supports mechanisms in which enolpyruvate is a true intermediate in the overall allylic substitution catalyzed by pyruvate kinase and confirms data showing the separation of enolization and phosphoryl transfer steps. Furthermore this result demonstrates the usefulness of in situ generated enolpyruvate for enzymatic studies.

References and Notes

(1) This work was supported by U.S. Public Health Service Grants GM-20940, CA-06927, and RR-05539, and by an appropriation from the Commonwealth of Pennsylvania.
(2) I. A. Rose, *J. Biol. Chem.*, **235**, 1170 (1960).
(3) G. W. Kosicki, *Biochemistry*, **7**, 4310 (1968).
(4) I. A. Rose, E. L. O'Connell, P. Noce, M. F. Utter, H. G. Wood, J. M. Willard, T. G. Cooper, and M. Benziman, *J. Biol. Chem.*, **244**, 6130 (1969).
(5) J. L. Robinson and I. A. Rose, *J. Biol. Chem.*, **247**, 1096 (1972).
(6) J. M. Willard and I. A. Rose, *Biochemistry*, **12**, 5241 (1973).
(7) I. A. Rose, E. L. O'Connell, and F. Solomon, *J. Biol. Chem.*, **251**, 902 (1976).
(8) M. J. Wimmer and I. A. Rose, *Annu. Rev. Biochem.*, **47**, 1031 (1978).
(9) H. G. Wood and G. K. Zwolinski, *CRC Crit. Rev. Biochem.*, **2**, 47 (1976).
(10) J. Moss and M. D. Lane, *Adv. Enzymol*, **35**, 321 (1971).
(11) J. Tetey and F. Lynen, *Biochem. Z.*, **342**, 256 (1965).
(12) A. S. Mildvan and M. C. Scrutton, *Biochemistry*, **6**, 2978 (1967).
(13) D. J. Prescott and J. L. Rabinowitz, *J. Biol. Chem.*, **243**, 1551 (1968).
(14) D. J. Kuo and I. A. Rose, unpublished results.
(15) I. A. Rose, *J. Biol. Chem.*, **245**, 6052 (1970).
(16) F. A. L. Anet, *J. Am. Chem. Soc.*, **82**, 994 (1960).
(17) O. Gawron, A. L. Glaid III, and T. P. Fondy, *J. Am. Chem. Soc.*, **83**, 3634 (1961).
(18) Y. F. Cheung, C. H. Fung, and C. Walsh, *Biochemistry*, **14**, 2981 (1975).

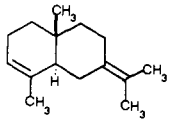
Donald J. Kuo, Irwin A. Rose*
The Institute for Cancer Research
The Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111
Received June 5, 1978

An Intramolecular Diels–Alder Route to Eudesmane Sesquiterpenes

Sir:

The steam volatile oil of hops[1] contains about 15 sesquiterpene hydrocarbons, one of which has been identified as selina-3,7(11)-diene (**1**).[2] This material has never been synthesized and represents a widespread group of compounds known as the eudesmane sesquiterpenes.[3] In general, synthetic

0002-7863/78/1500-6289$01.00/0

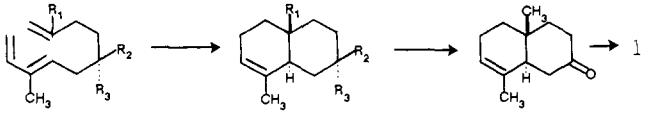


1

approaches to substituted decalins of this type rely on the classical Robinson annelation.[4-6] We report here our method of design based on the intramolecular Diels-Alder reaction.[7]

From a strategic point of view the construction of *two* six-membered rings simultaneously from an acyclic precurser has an advantage of convergence. Invariably, the methods[5,6] reported to date are multistage schemes requiring stepwise introduction of substituents. Our studies regarding the scope of a Diels-Alder construction had to address two questions: (1) what will be the stereochemistry of the ring fusion and (2) will introduction of an *angular methyl group* be tolerated?

We have recently reported[8] a convenient route to trisubstituted acyclic *E* 1,3-dienes such as **2a-e** suitably constituted



2a. $R_1=R_2=R_3=H$
b. $R_1=R_2=H, R_3=OH$
c. $R_1=CH_3, R_2=R_3=H$
d. $R_1=CH_3, R_2=H, R_3=OH$
e. $R_1=CH_3, R_2=H, R_3=OTMS$

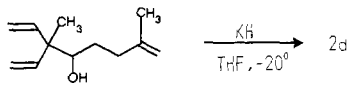
3a. $R_1=R_2=R_3=H$
b. $R_1=R_2=H, R_3=OH$
c. $R_1=R_3=H, R_2=OH$
d. $R_1=CH_3, R_2=R_3=H$
e. $R_1=CH_3, R_2=H, R_3=OH$
f. $R_1=CH_3, R_2=OH, R_3=H$

4

for intramolecular Diels-Alder cyclizations. Triene **2a** was prepared[8a] from 1-iodo-4-pentene and 3-methylpentadienyllithium and the mixture of regioisomers (1:1) separated by preparative GLC. Compound **2a** underwent stereospecific cyclization to **3a** (95% yield) at 160 °C. No cis compound could be detected.[9,10] Next the corresponding alcohol **2b** was prepared.[8a] Again the Diels-Alder reaction occurred smoothly to yield 24% **3b** (mp 85-86 °C) and 33% **3c**[11] after column chromatography. Thus the presence of the substituent on C-7 does not perturb the stereochemical control of the trans ring fusion. The major question remains however: will the intramolecular Diels-Alder tolerate the introduction of an angular methyl group? There are no reported cases of an intramolecular Diels-Alder reaction which generates a perhydronaphthalene with an angular methyl group.[12]

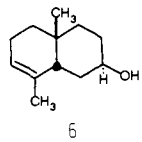
Hydrocarbon **2c** was prepared in the usual way.[8a] Thermolysis of **2c** in toluene at 190 °C gave an ~30% yield of compound **3d** (angular methyl, δ 0.80 (3 H, s); vinyl H, 5.25 (1 H, s)) whose IR spectrum matched that reported.[13] Thus the introduction of the angular methyl group and the trans ring fusion[14] can be accomplished in one step.

Application of this method to the synthesis of (±)-selena-3,7-diene (**1**) is now described. Reaction of 3-methylpentadienyllithium with 4-methyl-4-pentenal gave a 50% yield of **2d**[16] and a 45% yield of regioisomer **5**[8a] which could be isomerized



5

with KH to **2d** in 66% yield.[8b] Compound **2d** decomposed on heating; however, the silyl ether **2e** in toluene at 200 °C (100 h) underwent smooth Diels-Alder cyclization (95% GC yield) to give after hydrolysis a mixture of 34% trans-axial **3e**[17] (mp 116 °C), 54% trans-equatorial **3f**,[18] and 5% cis-equatorial **6**.[19] Oxidation of this mixture gave ketone **4**[20] (bp ~ 40 °C at 0.4 mm) in 89% yield (94:6 trans:cis). The overall yield of ketone **4** from 4-methyl-4-pentenal is 35%. Treatment[21] of **4** with

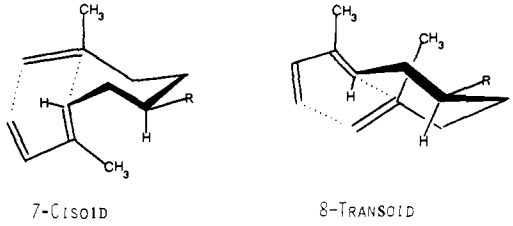


6

CBr_4/Ph_3P, followed by Me_2CuLi, gave (±)-selena-3,7(11)-diene (**1**) in 75% yield.[22]

Since many members of the eudesmane class differ only in constitution of the C-7 side chain, the route via **2d** yields a synthetic precurser of maximum flexibility. Compound **4** will undoubtedly be useful for the production of a number of α-eudesmane sesquiterpenes.

Obtention of trans-fused material in this series can be understood by noting that, in the transition state leading to cis ring fused material (**7**), there is a severe nonbonded interaction



7-Cisoid

8-Transoid

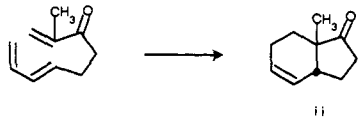
between the vinylic methyl on the diene and an axial hydrogen at C-7.[23] This interaction is absent in the transition state leading to trans-fused compounds **8**.[24]

In summary, we have shown by this work that the stereospecific construction of terpenes *with an angular methyl* is possible via the intramolecular Diels-Alder reaction. We are currently exploring applications of such strategy to similar molecules in the diterpene and steroid fields.

Acknowledgments. We thank the Research Corporation, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Institutes of Health (GM 24438) for financial support.

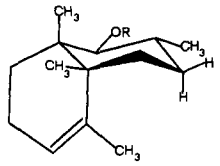
References and Notes

(1) R. Stevens, *Chem. Rev.*, **67**, 19 (1967).
(2) (a) R. G. Buttery, R. E. Lundin, and L. Ling, *Chem. Ind. (London)*, 1225 (1966); (b) R. D. Hartley and C. H. Fawcett, *Phytochemistry*, **8**, 637 (1969).
(3) T. K. Devon and A. I. Scott, "Handbook of Naturally Occurring Compounds: Terpenes", Vol. II, Academic Press, New York, N.Y., 1972.
(4) (a) R. E. Gawley, *Synthesis*, 777 (1976); (b) M. E. Jung, *Tetrahedron*, **32**, 3 (1976).
(5) The substantial synthetic activity in the eudesmane area has been reviewed: (a) J. M. Mellor, *Q. Rev., Chem. Soc.*, **18**, 270 (1964); (b) J. ApSimon, "The Total Synthesis of Natural Products", Vol. 2, Wiley, New York, N.Y., 1973, pp 282-330.
(6) Recent work has been based on introduction of the angular methyl via cuprate additions: (a) J. W. Huffman and M. L. Mole, *J. Org. Chem.*, **37**, 13 (1972); (b) R. G. Carlson and E. G. Zey, *ibid.*, **38**, 2468 (1972); (c) R. B. Miller and R. D. Nash, *ibid.*, **38**, 4424 (1973); (d) G. H. Posner and G. L. Loomis, *ibid.*, **38**, 4459 (1973).
(7) W. Oppolzer, *Angew. Chem., Int. Ed. Engl.*, **16**, 10 (1977).
(8) (a) S. R. Wilson, K. M. Jernberg, and D. T. Mao, *J. Org. Chem.*, **41**, 3209 (1976); (b) S. R. Wilson, D. T. Mao, K. M. Jernberg, and S. T. Ezmirly, *Tetrahedron Lett.*, 2559 (1977).
(9) All new compounds possessed spectral and analytical data consistent with the assigned structures. NMR spectra were determined on a Varian HR-220 at 220 MHz.
(10) The structure of **3a** was confirmed by spectral comparison with authentic trans material prepared from 1-methyl-1(9)-octal-2-one and by identical retention times on a 50 m × 0.25 mm glass capillary column (UCON 50-HB-2000). A small peak (<5%) at longer retention time could be the cis isomer.
(11) Both **3b** and **3c** were converted via known methodology to **3a**. In addition, the structure of **3b** was confirmed by a single-crystal X-ray diffraction experiment. Compound **3b** crystallized in the space group *P*1 with four molecules in the unit cell. Cell constants at −130 °C are a = 11.455 (7), b = 12.220 (7), c = 8.218 (5) Å; α = 119.29 (1), β = 81.16 (3), γ = 95.32 (3)°. Crystallographic data for this paper may be obtained in microfiche form for $2.50 from the Chemistry Library, Indiana University, Bloomington, Ind. 47401. Refer to J. C. Huffman, Indiana University, Molecular Structure Center Report No. 7806, 1978.
(12) (a) The intramolecular cyclization of i → ii occurs at 190 °C: J. J. S. Bajorek and J. K. Sutherland, *J. Chem. Soc., Perkin Trans. 1*, 1559 (1975). (b) For related cyclizations, see T. Kametani, Y. Hiral, F. Satoh, and K. Fukumoto, *J. Chem. Soc., Chem. Commun.*, 16 (1977); T. Kametani, M. Tsubuki, Y.



ii

Shiratori, Y. Kato, H. Ihara, and K. Fukomoto, *J. Org. Chem.*, **42**, 2672 (1977).
(13) J. A. Marshall and A. R. Hochstetler, *J. Am. Chem. Soc.*, **91**, 648 (1969).
(14) Control of the trans geometry is often lacking in eudesmane synthesis owing to the very small energy difference between cis and trans in this series.[15] The only really successful control of trans ring geometry is seen in routes involving Birch reduction.[5]
(15) A. Ross, P. A. Smith, and J. L. Coke, *J. Org. Chem.*, **26**, 2096 (1961).
(16) IR (film) 3330 cm^{-1}; NMR ($CDCl_3$) δ 6.38 (1 H, dd, J = 10 Hz, J = 17.5 Hz), 5.5 (1 H, t, J = 8 Hz), 4.7–5.25 (4 H, m), 3.65 (1 H, m), 1.95–2.22 (4 H, m), 1.5–1.8 (9 H, m).
(17) IR (KBr) 3250 cm^{-1}; NMR ($CDCl_3$) δ 5.3 (1 H, br s), 4.14 (1 H, m), 1.57 (3 H, s), 1.14–2.45 (12 H, m), 0.80 (3 H, s).
(18) IR (film) 3360 cm^{-1}; NMR ($CDCl_3$) δ 5.32 (1 H, br s), 3.59 (1 H, m), 1.60 (3 H, s), 1.70–2.05 (12 H, m), 0.82 (3 H, s).
(19) Compound **6** could not be isolated in pure form; however, its presence was inferred by the angular methyl at δ 0.91 and –CH–O at 3.80 in the NMR spectrum of mixtures.
(20) IR (film) 1720 cm^{-1}; NMR ($CDCl_3$) δ 5.40 (1 H, br s), 1.85–2.70 (8 H, m), 1.1–2.7 (6 H, m), 1.10 (3 H, s).
(21) G. H. Posner, G. L. Loomis, and H. S. Sawaya, *Tetrahedron Lett.*, 1373 (1975).
(22) Synthetic **1** possessed IR, NMR, and mass spectra identical with those of the natural material.[2]
(23) The X-ray structure of a cis-fused terpenoid iii related to **7** has been reported: G. Saucy, R. E. Ireland, J. Bordner, and R. E. Dickerson, *J. Org. Chem.*, **36**, 1195 (1971). The proximity (3.49 Å) of the vinylic methyl to C-7 can be clearly seen in the stereopair (Figure 1) in that paper, even though the A ring exists as a half-chair in the crystal structure, while the Diels–Alder transition state is a boat. Flipping from a boat to a half-chair greatly relieves the nonbonded interaction in question (3.5 Å vs. 3.0 Å in models).



iii (R=*p*-BROMOBENZOATE)

(24) Indirect evidence for transition states **7** and **8** is available from consideration of two points. First, the ratios of equatorial to axial alcohols **3c/3b** and **3f/3e** are 1.4:1 and 1.6:1, respectively. These ratios are about what are expected (based on known *A* values) for a transition state in which ring B is chair-like. Second, the lack of any detectable axial cis-fused alcohol is consistent with transition state **7** in which such an axial substituent would produce even more severe nonbonded interactions.

Stephen R. Wilson,* David T. Mao
Contribution No. 3094
Department of Chemistry, Indiana University
Bloomington, Indiana 47401
Received May 9, 1978

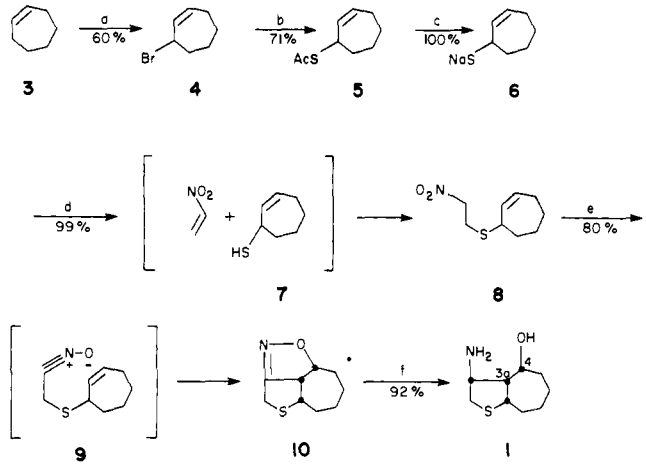
Use of Intramolecular [3 + 2] Cycloaddition Reactions in the Synthesis of Natural Products. A Stereospecific Synthesis of (±)-Biotin from Cycloheptene

Sir:

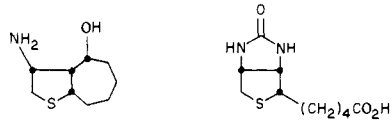
Reactions generally classified as 1,3-dipolar cycloadditions have been extensively employed in the synthesis of a diverse array of heterocyclic compounds.[1] However, this reaction mode has been alloted a more limited role in the preparation of natural products.[2] This is surprising since the cycloadditions are not only ring-forming reactions but also proceed with a high degree of stereoselectivity.[3]

We have utilized an intramolecular [3 + 2] cycloaddition reaction of an olefinic nitrile oxide in the stereospecific synthesis of the key amino alcohol **1**, which was converted in five subsequent steps to (±)-biotin (**2**).[4]

Scheme I



[a] a: NBS, AIBN, CCl_4, reflux, 1.5 h. b: AcSH, CH_3CN, Et_3N, 0 °C, 3 h. c: NaOEt/EtOH, reflux, 15 min. d: $NO_2CH_2CH_2OAc$, EtOH, 0 °C, 3 h. e: PhNCO, PhH, Et_3N (cat.), 25 °C, 24 h. f: $LiAlH_4$, Et_2O, reflux, 4 h.



1 2

Allylic bromination of cycloheptene **3** with NBS[5] and subsequent treatment of the 3-bromo product **4**[6] with thiolacetic acid yielded the desired thiol ester **5**, bp 64–65 °C (0.25 mm) (Scheme I), serving to introduce the requisite sulfur atom at an early stage. The mercaptide **6**, generated in situ with ethanolic sodium ethoxide, was treated with 1 equiv of 1-nitro-2-acetoxyethane,[7] a process which presumably generated nitroethylene and the mercaptan **7**. These intermediates then underwent a Michael reaction to afford the nitro olefin **8** (IR 1640 (C=C), 1555, 1378 cm^{-1} (NO_2); *m/e* 201 (M^+) in virtually quantitative yield. Treatment of this nitro compound with phenyl isocyanate led directly to the novel tricyclic adduct **10** (IR 1717 cm^{-1} (C=N), *m/e* 183 (M^+)) obtained stereospecifically in high yield as a colorless oil. This result implicates the intermediacy of an intramolecular [3 + 2] cycloaddition of the nitrile oxide **9**. Although only two of the three ultimate stereocenters of biotin were created in this step, the third was stereospecifically introduced in the desired cis configuration by $LiAlH_4$. This reagent not only cleaved the N–O bond of the tricyclic adduct **10** but also reduced the imino functionality. Hydride delivery occurred from the less hindered convex side[8] of the cup-shaped structure and led directly to the desired amino alcohol **1** (IR 3400 (OH), 3200–3350 cm^{-1} (NH_2)), characterized as its crystalline hydrochloride (IR 3100 cm^{-1} (NH_3^+); *m/e* 187 (M^+), mp 192–193 °C). This straightforward sequence of reactions allowed the preparation of pure **1** in an overall yield of 73% based on the thiol ester **5**.

Further elaboration of **1** in the direction of biotin required scission of the C(3a)–C(4) bond with insertion of a nitrogen atom attached to C(3a), as well as an elevation of C(4) to the oxidation state of an acid. To this end, the amino alcohol **1** was converted to the ketone **12** (IR 1703 cm^{-1} (ketone), mp 102–103 °C) via the intermediate urethane alcohol **11** (IR 3510 (OH), 3330 cm^{-1} (NH), mp 109–110 °C) (Scheme II). At this point, the ruinous possibility of an epimerization at C(3a) to the thermodynamically more stable trans fused 5,7 system had to be ruled out. Treatment of our pure all cis ketone with sodium acetate in refluxing ethanol quantitatively converted it to the trans isomer **12a**, verifying our structural assignment.[9] Treatment of the ketone **12** with hydroxylamine

0002-7863/78/1500-6291$01.00/0