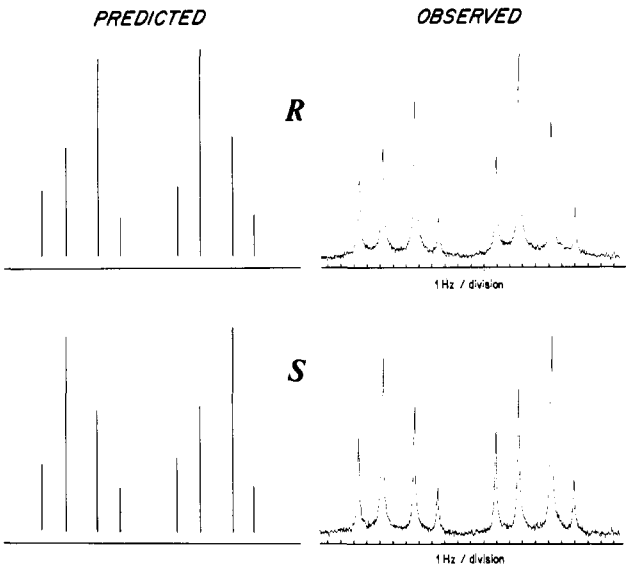

Figure 3. Predicted and observed ^{31}P NMR spectra of the mixtures of syn and anti cyclic triesters derived from labeled samples of 1-phospho-(*S*)-propane-1,2-diols that are *R* or *S* at phosphorus. (Spectrum of the *S* epimer, 200 transients; *R* epimer, 291 transients.)

in the synthesis. This knowledge permits one to predict the intensities of the eight lines in the ^{31}P NMR spectrum, four of which derive from the syn diastereoisomer and four from the anti diastereoisomer. The predicted spectra[12] are shown in Figure 3, along with the actual spectra[14] for the cyclic triesters derived from *R* and *S* 1-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol, synthesized independently.[15] The observed ^{18}O isotopic shifts (upfield from the ^{16}O,^{16}O compound, which gives the downfield signal of each set of four) are 0.018 ppm for the compounds containing one singly bonded ^{18}O (**6** and **8**), 0.043 ppm for the compounds containing one doubly-bonded ^{18}O (**3** and **9**), and 0.060 ppm for the materials containing two exocyclic ^{18}O atoms.

The spectra in Figure 3 show that a complementary pattern of eight peaks is obtained from the cyclic triesters obtained by ring closure and methylation of the two chiral phosphopropanediols. The agreement between the predicted and observed spectra makes the assignment of the absolute configuration at phosphorus unambiguous. Signal integration suggests that the *S* epimer contains about 82% of the *S* material and the *R* epimer contains about 76% of the *R* compound, though the imprecision of such integration makes these only approximate values.

It is therefore clear that high-resolution ^{31}P NMR offers a straightforward and experimentally simple method for the determination of the absolute configuration of [^{16}O,^{17}O,^{18}O]phosphate monoesters. The method is general, since we have already demonstrated the practicality of transferring a phosphoryl group from any location to (*S*)-propane-1,2-diol with retention of configuration.[2,3,4] Although less quantitative than our earlier approach,[1] the NMR method avoids both the need to separate the syn and anti diastereoisomers of the cyclic triesters and the need for linked-scan metastable ion mass spectrometry.

Acknowledgment. We are especially grateful to Drs. Ben Bangerter, Eric Fossel, and David Ruben for advice and help, to Peter Ziegler Esq. and Bruker Instruments, Inc. for the final ^{31}P NMR spectra, to the National Institutes of Health and Merck, Sharp & Dohme for support, and to Dr. Mildred Cohn for her enthusiastic espousal of the NMR approach, even before (1977) the mass spectrometric method had worked.

(17) National Science Foundation Predoctoral Fellow.

Stephen L. Buchwald,[17] Jeremy R. Knowles*

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

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Bioorganic Synthesis and Absolute Configuration of Faranal

Sir:

Faranal, a trail pheromone of the pharaoh ant, has a unique structure which is reminiscent of a juvenoid and is interesting from a biosynthetic point of view. The structure was first reported to be **6a** or its antipode (**6b**),[1] but later was revised as **7a** or its antipode (**7b**).[2] Since the pheromone is obtainable only in such a small quantity that no optical rotation datum is available, asymmetric synthesis of **7a** and **7b** by an unambiguous route and the comparison of their biological activity would be desirable to determine the absolute configuration. Farnesyl pyrophosphate synthetase is a promising candidate for an agent of central importance for this purpose, because we have shown that this enzyme can synthesize stereospecifically the *S* or *R* enantiomer of 4-methylfarnesyl pyrophosphate, depending on whether it is supplied with geranyl pyrophosphate and (*E*)-**1a** or (*Z*)-3-methylpent-3-enyl pyrophosphate (**1b**) as substrate.[3,4] This paper reports an application of biochemical systems in the asymmetric synthesis of the stereoisomers of faranal, leading to the conclusion that the absolute configuration of the natural faranal is 3*S*,4*R*.

Enzymatic condensation of **1a** or **1b** with homogeranyl pyrophosphate (**2**) obtained by the phosphorylation of 2(*E*),6(*Z*)-3,7-dimethylnona-2,6-dien-1-ol[5] was carried out on a large scale

(12) The predicted spectra are calculated on the basis that the (*S*)-2-benzylpropane-1,2-diol used in the synthesis[1] had 82.8% enantiomeric excess, as determined by the separation and quantitation (A_{254}) of the diastereoisomeric esters of (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543), that the isotopic composition of the peripheral oxygen atoms of the *R* and *S* phospho compounds was as in ref 11, and that the ratio of syn to anti cyclic triesters was 0.95 ± 0.05 (from integration of the two sets of four lines of the ^{31}P NMR spectra[13]).

(13) No attempt was made to correct for any NOE differences between the syn and anti isomers.

(14) Fourier transform ^{31}P NMR spectra were obtained through the kindness of P. Ziegler of Bruker Instruments, Inc. A Bruker WM-250 instrument was used[10] at 101.27 MHz with a deuterium field lock. A spectral width of 1000 Hz was used with a pulse width of 22 μs and an acquisition time of 8.2 s. The sample [approximately 140 μmol of the bis(tri-*N*-octylammonium) salt of *R* or *S* 1-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol] was cyclized by using diphenylphosphorylimidazole[1,15] (125 μmol) in CH_2Cl_2. After workup,[1] the cyclic diesters were dissolved in CD_3CN/CH_3CN (3:7, v/v) and esterified with excess ethereal diazomethane. The resulting solution of cyclic triesters was concentrated to 2 mL and filtered through glass wool into a precision 10-mm NMR tube under dry argon.

(15) The method described in ref 1 was used, the configuration at phosphorus being determined simply by the order of introduction of ^{17}O and ^{18}O.

(16) These figures are drawn on the hypothetical basis that the oxygen isotopic enrichment at the indicated positions is 100% (see text).

(1) Ritter, F. J.; Brüggemann-Rotgans, I. E. M.; Verwiel, P. E. J.; Persoons, C. J.; Talman, E. *Tetrahedron Lett.* **1977**, 2617-2618.

(2) Ritter, F. J.; Brüggemann-Rotgans, I. E. M.; Verwiel, P. E. J.; Talman, E.; Stein, F.; La Brijn, J.; Persoons, C. J. *Proc. Int. Congr. Int. Union Study Soc. Insects, 8th* **1977**, 41-43. As the revision was only mentioned in the erratum of ref 2 and in an unpublished TNO report,[10] the evidence to rule out **6a** and **6b** will be summarized here. Ozonolysis of faranal yields a dimethyl dialdehyde with a retention time equal to that of the dimethyl dialdehyde formed from *cis*-1,2-dimethyl-4-cyclohexene and different from that of the dimethyl dialdehyde formed from *trans*-1,2-dimethyl-4-cyclohexene (Figure 1). Moreover, a gas chromatogram of an ozonolyzed mixture of faranal and *cis*- and *trans*-1,2-dimethyl-4-cyclohexene showed that only the peak of the dialdehyde formed from the cis isomer was increased. Thus, it could be concluded that faranal has either the 3*R*,4*S* structure (**7a**) or the 3*S*,4*R* structure (**7b**). The racemic mixture of these two enantiomers was synthesized, starting from *cis*-1,2-dimethyl-4-cyclohexene, by a method described before.[11] The synthetic racemate had identical spectroscopic and chromatographic properties as the isolated pheromone and was biologically active.

(3) Koyama, T.; Ogura, K.; Seto, S. *J. Am. Chem. Soc.* **1977**, *99*, 1999-2000.

(4) Koyama, T.; Saito, A.; Ogura, K.; Seto, S. *J. Am. Chem. Soc.* **1980**, *102*, 3614-3618.

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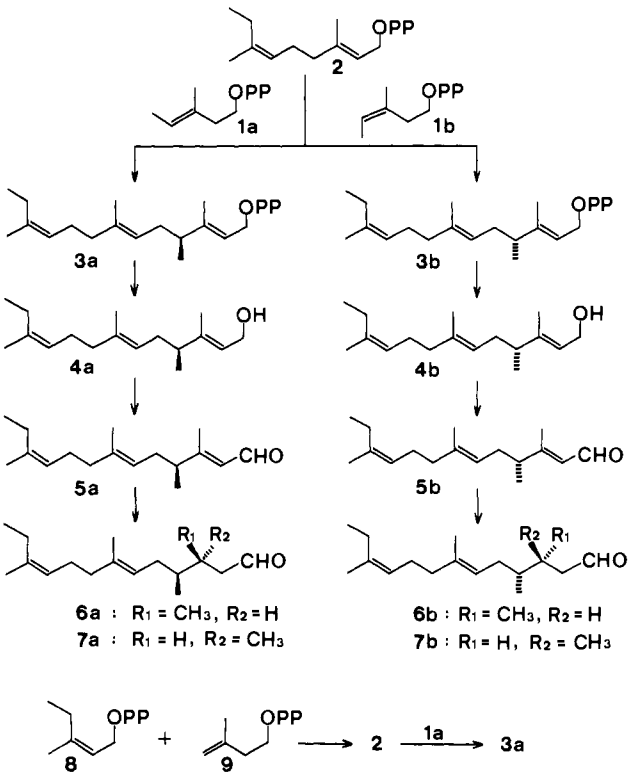
as follows. The incubation mixture contained, in a final volume of 500 mL, 10 mmol of Tris-HCl buffer, pH 7.7, 5 mmol of $MgCl_2$, 5 mmol of 2-mercaptoethanol, 1.5 mmol of 1,4-dithiothreitol, 37.5 μmol of **2**, 25 μmol of **1a**, and 50 mg of farnesyl pyrophosphate synthetase purified from pig liver.[6] After the incubation at 37 °C for 12 h, the mixture was treated with alkaline phosphatase to hydrolyze the pyrophosphate esters, and the products were extracted with petroleum ether. The hydrolysates showed two peaks on GLC–mass spectrometry, one corresponding to homogeraniol recovered from **2** and the other to the condensation product. The mass spectrum of the latter exhibited a molecular ion at *m/e* 250, corresponding to dimethylfarnesol ($C_{17}H_{30}O$) and fragment ions at 232 (M − 18), 219 (M − 31), 151 (M − 18 − 81), 149 (M − 18 − 83), and 83 (C_6H_{11}) which was the base peak. The alcohol was purified by TLC and high-pressure liquid chromatography (high-pressure LC) (yield 4.5 mg, 30% based on **1a**) and was subjected to spectral measurements. The NMR spectrum showed peaks at δ 0.96 (t, 3 H), 0.99 (d, 3 H), 1.56 (s, 3 H), 1.59 (s, 3 H), 1.63 (s, 3 H), 2.00 (m, 9 H), 3.98 (d, 2 H), 4.94 (m, 2 H), and 5.28 (t, 1 H). The ORD spectrum showed a negative curve ($[\alpha]_{300}$ −22.4°) as expected.[4] These results indicate that the alcohol is 4(*S*)-2(*E*),6(*E*),10(*Z*)-3,4,7,11-tetramethyltrideca-2,6,10-trien-1-ol (**4a**) derived from its pyrophosphate (**3a**).

Compound **3a** was also obtained conveniently by sequential enzymatic reactions by using (*Z*)-3-methylpent-2-enyl pyrophosphate (**8**), isopentenyl pyrophosphate (**9**), and **1a** as substrates. To a reaction mixture containing **2** as a product of preincubation of **8** and **9** with farnesyl pyrophosphate synthetase for 30 min were added **1a** and fresh enzyme, and the incubation was continued for another 30 min. The GLC–mass spectrometric analysis of the alcohols derived from the products of this incubation revealed that **4a** was formed as a major product with a concomitant formation of 3,7,11-trimethyltrideca-2,6,10-trien-1-ol and 3,4,7,8,11-pentamethyltrideca-2,6,10-trien-1-ol.

Treatment of **4a** with active MnO_2 gave the corresponding aldehyde (**5a**)[7] which showed a positive optical rotation, $[\alpha]_{300}$ +49.8°, as expected from our previous observation.[4] The remaining task to obtain faranal is a selective reduction of the 2,3-double bond of **5a**. For this purpose, the method of Ojima et al.[8] was applied. A benzene solution of 1.5 mg of **5a** and 4.5 mg of geranylgeranial added as a carrier[9] was treated with triethylsilane in the presence of $(Ph_3P)_3RhCl$, and the resulting silyl enol ethers were hydrolyzed to give a mixture of the 2,3-dihydro derivatives, from which 260 μg of a mixture of 3(*S*),4(*S*)- (**6a**) and 3(*R*),4(*S*)-faranal (**7a**) was separated by TLC and high-pressure LC. The mass spectrum of the faranal thus obtained (*m/e* 250, 232, 221, 203, 193, 175, 153, 137, 123, 107, 95, 83, 55) was identical with that of the natural product. Similarly, the enzymatic reaction starting from **2** and **1b** afforded 700 μg of 4(*R*)-2(*E*),6(*E*),10(*Z*)-3,4,7,11-tetramethyltrideca-2,6,10-trien-1-ol (**4b**), from which the other pair of the diastereomers, 3(*R*),4(*R*)- (**6b**) and 3(*S*),4(*R*)-faranal (**7b**) were also obtained by the same method as described for the 4*S* isomers (Scheme I).

The bioassay carried out with pharaoh ants clearly discriminated between these two series of synthetic faranals. The ants followed a trail of 0.5 ng/cm of 4(*R*)-faranals whereas none of them followed a comparable trail of 4(*S*)-faranals. When compared directly in a choice test with trails of 0.05 ng/cm, the preference

Scheme I



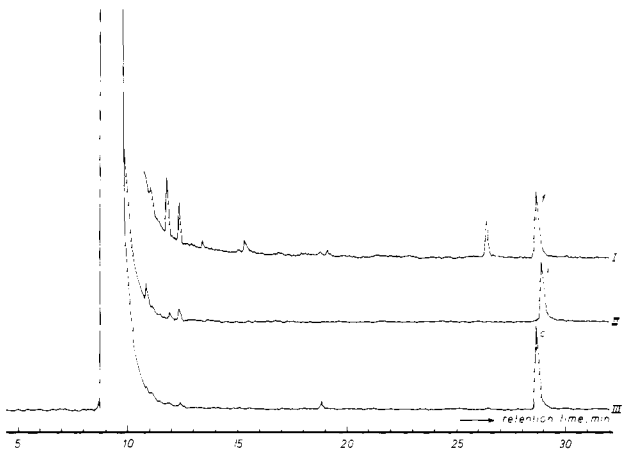


Figure 1. Gas chromatograms of the ozonolysis products of faranal (I), *trans*-1,2-dimethyl-4-cyclohexene (II), and *cis*-1,2-dimethyl-4-cyclohexene (III).

of the ants for the 4*R* isomers was quite unambiguous. The trail of the 4(*R*)-faranals was only slightly less active as compared with that of an equal concentration of the natural pheromone. Thus, it can be concluded that the absolute configuration of the pheromone is 3*S*,4*R* (**7b**) since the 3*R*,4*R* structure (**6b**) has been ruled out by the ozonolysis experiments.[2]

Furthermore, the gas chromatography of the 4(*R*)-faranals on a 90-m SE-30 capillary column resulted in the separation of the diastereomers, and the ratio of formation of **6b** and **7b**, the latter having a retention time equal to that of the isolated natural faranal, was estimated to be 2:11. Both isomers showed pheromone activity when tested separately. However, it took $^1/_2$ min before the ants followed the trail of **6b** whereas it took only a few seconds before they did so with **7b**. In a choice test with trails of equal concentration (about 0.05 ng/cm), almost all ants followed the trail of **7b**.

These results suggest that the 4*R* configuration is crucially important for the pheromone activity and that the stereospecificity of the enzymatic C–C bond formation is extremely high.

(5) Dahm, K. H.; Trost, B. M.; Röller, H. *J. Am. Chem. Soc.* **1967**, *89*, 5292–5294.
(6) Holloway, P. W.; Popják, G. *Biochem. J.* **1967**, *104*, 57–70.
(7) Yield 2.6 mg (58%). Mass spectrum: *m/e* 248 (M) 230, 219, 165, 83. NMR: δ 0.96 (t, 3 H), 1.00 (d, 3 H), 1.57 (s, 3 H), 1.63 (s, 3 H), 2.01 (m, 9 H), 2.09 (s, 3 H), 4.93 (m, 2 H), 5.69 (br d, 1 H), 9.79 (d, 1 H).
(8) Ojima, I.; Kogure, T.; Nagai, Y. *Tetrahedron Lett.* **1972**, 5035–5038.
(9) In the absence of this carrier, the reduction was not successful, but in the presence of it even only 20 μg of **5a** was successfully reduced.
(10) Brüggemann-Rotgans, I. E. M.; Ritter, F. J.; Verwiel, P. E. J.; Talman, E. "Isolatie, Identificatie en Biologische Activiteit van Faranal, een Spoorvolgstof van de Faraomier", Report CL 77/88 of the Central Laboratory TNO, Delft, Netherlands, 1977, pp 1–30.
(11) U.S. Patent 4146609, 1979.

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Masaki Kobayashi, Tanetoshi Koyama, Kyozo Ogura* Shuichi Seto

Chemical Research Institute of Non-Aqueous Solutions Tohoku University, Sendai 980, Japan

F. J. Ritter, I. E. M. Brüggemann-Rotgans

Netherlands Organization for Applied Scientific Research TNO, Division of Technology for Society Delft, The Netherlands

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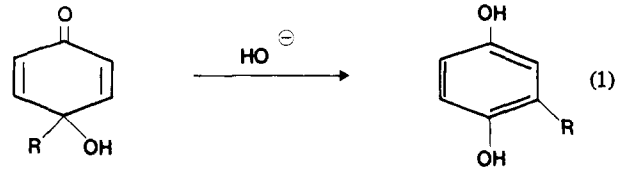
Conformational and Electronic Control of Anionic Rearrangements of 4-Hydroxycyclohex-2-en-1-ones

Sir:

4-Substituted 4-hydroxycyclohexa-2,5-dien-1-ones (**1**) have long been known to undergo rearrangement to 2-substituted hydroquinones (**2**) upon treatment with base.[1] This transformation,



(1)

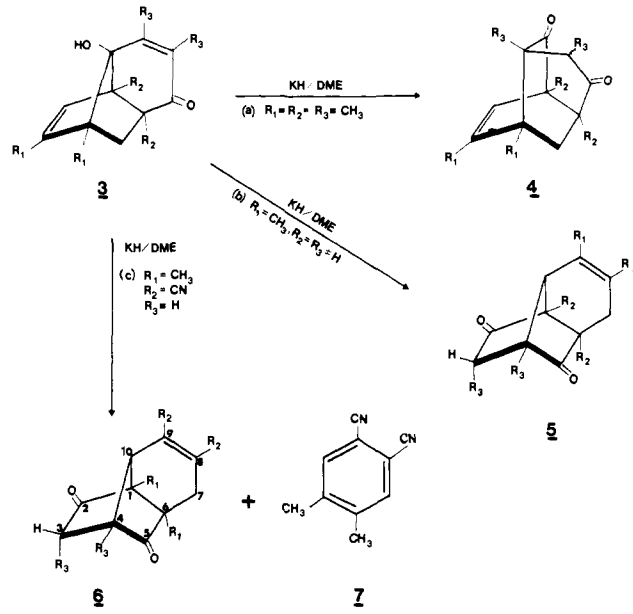
1 **2**

which may be termed a vinylogous acyloin rearrangement, presumably owes its driving force to the attainment of the hydroquinone product aromaticity.

We recently discovered what appeared to be a novel, nonaromatic version of this rearrangement, namely, the conversion of 4-hydroxycyclohexenone **3a** ($R_1 = R_2 = R_3 = CH_3$) into the twistane derivative **4** upon treatment with potassium hydride in dimethoxyethane (Scheme I).[2] However, in this communication, we report that (1) twistane formation is *not* a general process for compounds of general structure **3** when treated similarly, (2) alternative, highly unusual anionic rearrangements occur under these conditions, and (3) the divergent rearrangement pathways can be understood on the basis of the conformational and electronic effects exerted by the substituents present in **3**.

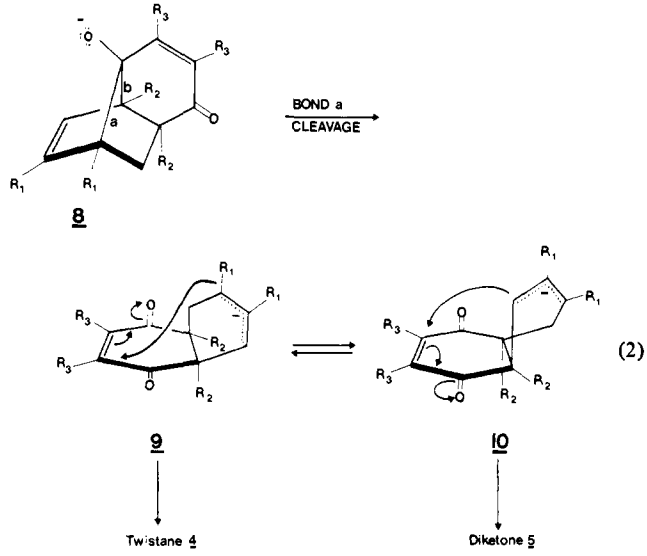
For example, treatment of substrate **3b**[3] ($R_1 = CH_3$, $R_2 = R_3 = H$) with potassium hydride in dimethoxyethane gave a 35% isolated yield (after recrystallization) of the diketone **5** (Scheme I).[3] Gas chromatography indicated that **5** constituted >95% of the total volatile products formed. Even more intriguingly, hydroxycyclohexenone **3c** ($R_1 = CH_3$, $R_2 = CN$, $R_3 = H$)[4] afforded diketone **6** (90% by GC, 34% isolated) plus dinitrile **7** (5% by GC, 2% isolated) when treated under similar conditions (Scheme I). Note that while products **5** and **6** have identical ring skeletons, they possess different substitution patterns, indicating they are formed by different mechanisms. The structure of **6** was deduced

Scheme I



from its spectral data,[5] and **7** is a known compound, mp 179-180 °C (lit.[6] mp 178-179 °C).

The divergent results for **3a**, **3b**, and **3c** can be nicely rationalized if one makes the unifying assumption that the initial step following proton removal in each case is cleavage of either bond a or bond b of alkoxide **8**. Whether bond a or bond b cleaves



(2)

depends in turn on the nature of R_2. When R_2 = CN as in **3c**, bond b cleaves for reasons of carbanion resonance stabilization whereas in the absence of this effect (R_2 = H or CH_3, compounds **3b** and **3a**, respectively) cleavage of bond a is intrinsically favored.

Cleavage of bond a leads to an allyl anion having conformation **9** which can close directly to the twistane product **4** via an internal

(1) (a) Bamberger, E. *Chem. Ber.* **1900**, *33*, 3600-3622. (b) Goodwin, S.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 179-185. (c) Nishinaga, A.; Itahara, T.; Matsuura, T. *Chem. Ber.* **1976**, *109*, 1530-1548.

(2) Greenhough, T. J.; Scheffer, J. R.; Trotter, J.; Wong, Y-F. *J. Chem. Soc., Chem. Commun.* **1979**, 933-934.

(3) The preparation and characterization of compounds **3b** and **5** are described in Scheffer, J. R.; Bhandari, K. S.; Gayler, R. E.; Wostradowski, R. A. *J. Am. Chem. Soc.* **1975**, *97*, 2178-2189.

(4) Scheffer, J. R.; Jennings, B. M.; Louwerens, J. P. *J. Am. Chem. Soc.* **1976**, *98*, 7040-7048.

(5) Compound **6**: mp 225-226 °C; UV (MeOH) λ_{max} 234 nm (ϵ 7900); IR (KBr) 1739 (C=O), 2232 cm^{-1} (CN); 270-MHz NMR (acetone-d_6) δ 0.95 (s, 3 H, CH_3), 1.17 (s, 3 H, CH_3), 2.30 (d, J = 18 Hz, 1 H, C(3) endo), 2.59 (d, J = 20 Hz, 1 H, one of C(7) methylenes), 2.77 (d, J = 20 Hz, 1 H, one of C(7) methylenes), 2.81 (dd, J = 18, 5 Hz, 1 H, C(3) exo), 3.40 (s, 1 H, C(10) methine), 3.59 (d, J = 5 Hz, 1 H, C(4) methine); mass spectrum parent (70 eV), m/e 240. Anal. Calcd for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.70; H, 5.19; N, 11.71. The location of the methyl groups in **6** follows from their NMR high-field chemical shifts and lack of coupling. The location of the nitrile groups is indicated by their red-shifted IR bands as compared to saturated nitriles. For example, the CN stretching frequency for **3c** is 2270 cm^{-1}.

(6) Mikhalenko, S. A.; Gladyr', S. A.; Luk'yanets, E. A. *Zh. Org. Khim.* **1972**, *8*, 341-343.

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