a dichroic ratio of 0.35.[26] If this in vivo observation is to be attributed to the P700, it may be interpretable in terms of exciton interations in a higher excited state than the lowest lying S_1 state in (Chl a·H_2O)$_2$. The present analysis has been specialized to the Q_y transition in Chl a.[27] For the Q_x transition the intensity ratio of the two exciton components remains 3 for the same ground-state nuclear configuration except that the long-wavelength component now becomes the dominant transition.[28]

The present work is an initial attempt at an optical characterization of (Chl a·H_2O)$_2$ in terms of a highly specific molecular model. The underlying assumptions implicit in the foregoing analysis are (A) that the A700 excitation spectrum in Figure 1 provides a faithful representation of the A700 absorption spectrum, and (B) that the A700 red band can be deconvoluted into two or less Gaussian exciton components. Assumption (A) seems to be reasonable in view of the comparison of the A700 spectrum in Figure 1 with the corresponding 121 K absorption spectrum displayed in Figure 2 of ref 10. The absence in the A700 excitation spectrum of the 672.5-nm absorption band, attributable to monomeric hydrated chlorophyll,[2,10] indicates that interaggregate excitation transfer is unimportant in the present case. Assumption B is consistent with the fact that no more than two exciton components can arise from a dimeric aggregate. In a dimeric Chl a–H_2O aggregate assuming the geometrical configuration of two neighboring units in the dihydrate polymer (Chl a·$2H_2O$)$_n$, only a single exciton component is expected[22a] because the transition dipole moments of the two Chl a molecules in this case are parallel to each other, i.e., $\theta = 0$.[23] The assumption of Gaussian behavior for the exciton bands is standard according to the theory of inhomogeneous line width broadening.

The two-component analysis given above has been based on the C_2-symmetrical dimer involving C-10 carbomethoxy C=O···H(H)O···Mg linkages. A somewhat analogous C_2-symmetrical model for (CHl a·H_2O)$_2$ invoking reciprocal C-9 keto C=O···H(H)O···Mg linkages[29] is expected to yield a predominantly one-component red band according to eq 4 because the transition moments of the Chl a molecules in this case are approximately antiparallel, i.e., $\theta = 180°$. We have recently prepared a 700-nm absorbing dimeric aggregate of pyroChl a in which the C-10 carbomethoxy carbonyl group is absent. The red band of this pyroChl a aggregate consists of a single Gaussian.[30]

Note Added in Proof. We note that Katz and co-workers, who, among others, have favored an unsymmetrical reaction center dimer (*Proc. Natl. Acad. Sci. U.S.A.*, **71,** 4897 (1974); *Biochem. Biophys. Comm.*, **71,** 671 (1976)) on the basis of a triplet esr signal that may be attributed to the inactive component P800 of the bacterial reaction center (see ref 9, p 283), now support a model equivalent in stoichiometry and symmetry to our model, and in bonding interactions to the Closs structure[29] (*Proc. Natl. Acad. Sci. U.S.A.*, **73,** 1791 (1976)).

Acknowledgment. This research was supported by the National Science Foundation (BMS 7411919).

References and Notes

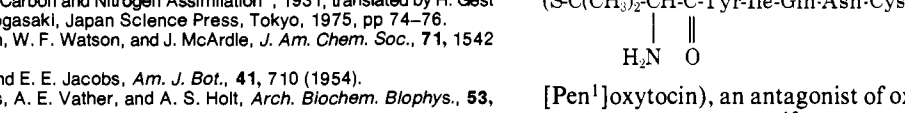
(1) K. Shibata, "Carbon and Nitrogen Assimilation", 1931, translated by H. Gest and R. K. Togasaki, Japan Science Press, Tokyo, 1975, pp 74–76.
(2) R. Livingston, W. F. Watson, and J. McArdle, *J. Am. Chem. Soc.*, **71,** 1542 (1949).
(3) A. S. Holt and E. E. Jacobs, *Am. J. Bot.*, **41,** 710 (1954).
(4) E. E. Jacobs, A. E. Vather, and A. S. Holt, *Arch. Biochem. Biophys.*, **53,** 228 (1954).
(5) G. Sherman and S. F. Wang, *Nature (London)*, **212,** 588 (1966).
(6) (a) E. K. Putseiko in "Elementary Photoprocesses in Molecules" Bertol'd S. Neporent, Ed., Consultant Bureau, New York, N.Y., 1968, p. 281; (b) Terenin, E. Putseiko, and I. Akimor, *Discuss. Faraday Soc.*, **27,** 83 (1959).
(7) (a) C. W. Tang and A. C. Albrecht, *J. Chem. Phys.*, **62,** 2139; **63,** 953 (1975); (b) C. W. Tang, F. Douglas, and A. C. Albrecht, *J. Phys. Chem.*, **79,** 2823 (1975); (c) C. W. Tang and A. C. Albrecht, *Mol. Cryst. Liq. Cryst.*, **25,** 53 (1974).
(8) For a comprehensive review of work prior to 1968, see, G. P. Gurinovich, A. N. Sevchenko, and K. N. Solovév, "Spectroscopy of Chlorophyll and Related Compounds", Chapter 9, available in a 1971 translation issued by the Division of Technical Information, U.S. Atomic Energy Commission, AEC-tr-7199.
(9) For a review of recent developments, see, F. K. Fong, "Theory of Molecular Relaxation: Applications in Chemistry and Biology", Wiley-Interscience, New York, N.Y., 1975, Chapter 9.
(10) F. K. Fong and V. J. Koester, *Biochim. Biophys. Acta*, **423,** 52 (1976).
(11) (a) F. K. Fong and V. J. Koester, *J. Am. Chem. Soc.*, **97,** 6888 (1975); (b) F. K. Fong, *ibid.*, **97,** 6890 (1975).
(12) N. Winograd, A. Shepard, D. H. Karweik, V. J. Koester, and F. K. Fong, *J. Am. Chem. Soc.*, **98,** 2369 (1976).
(13) B. Kok, *Biochim. Biophys. Acta*, **48,** 527 (1961).
(14) B. Ke, *Arch. Biochem. Biophys.*, **152,** 70 (1972).
(15) K. D. Phillipson, V. L. Sato, and K. Sauer, *Biochemistry*, **11,** 4591 (1972).
(16) (a) F. K. Fong, *J. Theor. Biol.*, **46,** 407 (1974); (b) *Proc. Natl. Acad. Sci. U.S.A.*, **71,** 3692 (1974); (c) *Appl. Phys.*, **6** (2), 151 (1975).
(17) V. J. Koester, J. S. Polles, J. G. Koren, L. Galloway, R. A. Andrews, and F. K. Fong, *J. Lumin.*, **12,** 781 (1976).
(18) F. K. Fong and N. Winograd, *J. Am. Chem. Soc.*, **98,** 2287 (1976).
(19) A. S. Davydov, "Theory of Molecular Excitons", McGraw-Hill, New York, N.Y., 1962.
(20) I. Tinoco, Jr., *Radiat. Res.*, **20,** 133 (1963).
(21) A detailed discussion of this calculation will be given elsewhere, V. J. Koester and F. K. Fong, *J. Phys. Chem.*, in press.
(22) For related exciton treatments that are not directly applicable to the (Chl a·H_2O)$_2$ model, see, for example, (a) E. G. McRae and M. Kasha, *J. Chem. Phys.*, **28,** 721 (1958); (b) R. M. Hochstrasser and M. Kasha, *Photochem. Photobiol.*, **3,** 317 (1964).
(23) H.-C. Chow, R. Serlin, and C. E. Strouse, *J. Am. Chem. Soc.*, **97,** 7230 (1975).
(24) Reference 9, p 282.
(25) (a) J. Breton and E. Roux, *Biochem. Biophys. Res. Commun.*, **45,** 557 (1971); (b) J. Breton, M. Michel-Villez, and G. Paillotin, *Biochim. Biophys. Acta*, **314,** 42 (1973); (c) J. Breton, E. Roux, and J. Whitmarsh, *Biochem. Biophys. Res. Commun.*, **64,** 1274 (1975).
(26) J. Breton, private communication, Jan 30, 1976.
(27) M. Gouterman and L. Stryer, *J. Chem. Phys.*, **37,** 2260 (1962).
(28) The validity of considering exciton interactions in excited electronic manifolds in terms of the ground state equilibrium nuclear configuration is based on the adiabatic principle of the separation of electronic and nuclear coordinates. See, for example, ref 9, Chapter 5.
(29) S. G. Boxer and G. L. Closs, *J. Am. Chem. Soc.*, **98,** 5406 (1976).
(30) F. K. Fong, V. J. Koester, L. Galloway, and L. Fetterman, *J. Am. Chem. Soc.*, submitted.

F. K. Fong,* V. J. Koester, J. S. Polles
Department of Chemistry, Purdue University
West Lafayette, Indiana 47907
Received February 18, 1976

Studies on the Molecular Association of Oxytocin and Related Compounds in Dimethyl Sulfoxide

Sir:

Numerous studies have been made on structural and conformational properties of peptide hormones and related compounds using high resolution NMR spectroscopy[1] and more recently carbon-13 NMR spectroscopy.[2] Among the peptide hormones which have received considerable attention are the neurohypophyseal peptides including oxytocin.[1,3] Most of these studies have been conducted at high peptide concentrations (25–250 mg/ml) where intermolecular interactions and association may affect derived parameters. We have studied the viscosity and proton FT-NMR of oxytocin and [1-penicillamine]oxytocin,



[Pen^1]oxytocin),

[Pen^1]oxytocin), an antagonist of oxytocin, and the carbon-13 T_1 values of specific ^{13}C-labeled atoms in oxytocin, as a function of concentration. Comparisons were made of dimethyl sulfoxide (Me_2SO) and aqueous solutions of these peptides. We present evidence which suggests that such interactions or associations may obtain in Me_2SO solutions.

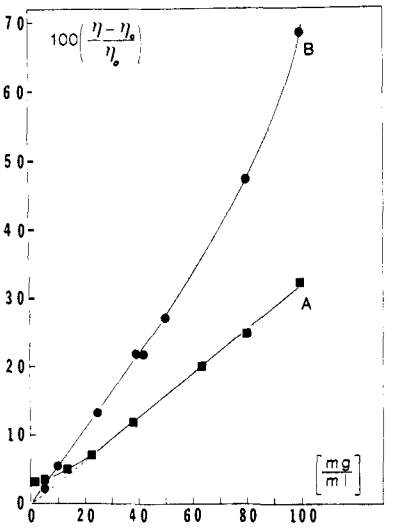


Figure 1. The change in the specific viscosity (×100) of oxytocin solutions as a function of concentration (A) in D_2O and (B) in $[^2H_6]Me_2SO$: η, viscosity of oxytocin solution; η_0, viscosity of the solvent. $\eta_0(Me_2SO)/\eta_0(D_2O) \simeq 2$.

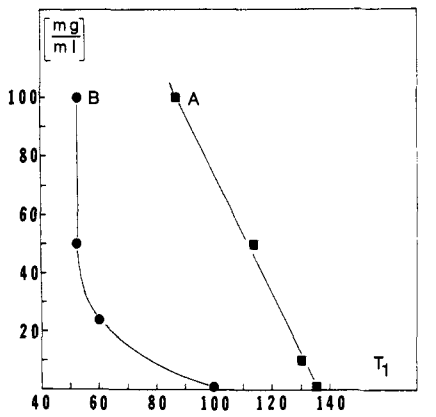


Figure 2. The change in T_1 (ms) of the 2-^{13}C carbon atom (90% ^{13}C-enriched) in the half-cystine-1 residue of [1-hemi[2-^{13}C]cystine]oxytocin (I) evaluated at different peptide concentration (mg/ml) in D_2O at pH 3.9 (A) and in $[^2H_6]Me_2SO$ (B). T_1 measurements were obtained on a Bruker WH-90 FT spectrometer using a 180°-τ-90° t pulse sequence, where t is at least five times T_1. The T_1 values have an experimental error of ±10–15%. The T_1 values at the various concentrations are (C, mg/ml (T_1, ms)): A, 100 (87), 50 (113), 10 (130), 1 (136); B, 100 (53), 50 (53), 25 (60), 1 (100).

Oxytocin and [Pen^1]oxytocin were synthesized and obtained in a highly purified form by procedures used in our laboratory.[5,6] [9-[2-^{13}C]glycinamide]oxytocin (I) and [1-hemi[2-^{13}C]cystine]oxytocin (II) (both 90% ^{13}C-enriched) were prepared by solid phase peptide synthesis.[7] pH values are direct pH meter (Radiometer) readings. T_1 measurements were obtained on a Bruker WH-90 FT spectrometer at 32 °C. Proton NMR dilution experiments were made on a Bruker HX-270 FT spectrometer. Viscosity measurements were made using a semimicro glass viscometer (Cannon: 1 ml). The viscometer was maintained in a water bath kept at a temperature of 32.0 °C. The flow times were determined with a stopwatch (±0.01 s) and were reproducible to ±0.5%. At least five different determinations were made for each point and in several cases using two different hormone solution preparations. No kinetic energy corrections were made since they are negligible.

Since Me_2SO is very hygroscopic, we minimized the uptake of water by tightly stoppering the viscometer containing the solution and placing it in a desiccator when viscosity measurements were not being made. Parallel experiments were run with Me_2SO alone to correct for the small amount of water which was picked up in the course of the experiments. By taking these precautions, highly reproducible values were obtained. The measured viscosity of the peptide solutions in Me_2SO is time dependent, especially at high concentrations, taking as much as several hours to reach equilibrium. These results are highly indicative[8] of an association of oxytocin in Me_2SO solution. Further evidence is provided by the change in viscosity of oxytocin solutions as a function of oxytocin concentration in $[^2H_6]Me_2SO$ (B) and in D_2O (A) shown in Figure 1. In D_2O solutions there was no change in viscosity as a function of time, and no significant change was found at pH 7 and 3.8. In D_2O the change in viscosity with concentration is linear except for a slight deviation at low concentrations (about 10 to 1 mg/ml) which was highly reproducible. Similar experiments were run with [Pen^1]oxytocin (not shown), and in D_2O a linear change in viscosity was observed over the entire concentration range. On the other hand, the specific viscosity of oxytocin solutions in Me_2SO (Figure 1) as a function of concentration is nonlinear, and shows a considerably greater magnitude of change than that found in aqueous solution over the same concentration range. Similarly a plot of η_{sp}/C vs. C (not shown) for a D_2O solution of oxytocin is linear, but this plot for a Me_2SO solution of oxytocin is nonlinear and does not agree with the Huggin's equation.[8]

The T_1 of the labeled half-cystine-1 carbon in [1-hemi[2-^{13}C-cystine]oxytocin (I) (measured at different concentrations) in D_2O at pH 3.8 (A) and in $[^2H_6]Me_2SO$ (B) is shown in Figure 2. In both cases the values at 100 mg/ml were taken from the literature.[9,10] In D_2O the T_1 values vary linearly with the concentration and closely parallel the observed viscosity changes. In $[^2H_6]Me_2SO$, on the other hand, a very marked nonlinearity of the T_1 values as a function of peptide hormone concentration is observed, and the effect does not parallel the viscosity changes. Similar experiments were run in D_2O using [9-[2-^{13}C]glycinamide]oxytocin (II), and gave similar results. Accurate T_1 studies of II in $[^2H_6]Me_2SO$ could not be made since one of the $[^2H_6]Me_2SO$ carbon-13 peaks overlaps with the labeled glycinamide carbon in oxytocin and this severely interferes with the determination of the T_1.

In the case of nonchemically exchanging species, the T_1 values which are experimentally obtained should be inversely proportional to the viscosity[11,12] (which is an implicit function of the concentration). The results we obtained show that the carbon-13 T_1 value for the half-cystine-1 2-carbon in II is concentration dependent in a way which cannot be accounted for by simple changes in viscosity. Alternatively if an intermolecular association or interaction of oxytocin in Me_2SO solution obtains, the observed T_1 will depend on the intrinsic T_1 of each state of association or interaction and on their respective lifetimes.[13] A nonlinear relationship of concentration to T_1 can be obtained in these circumstances, and consequently a reasonable explanation for the experimental behavior of T_1 in Me_2SO is a chemical exchange originating from an intermolecular association or interaction.

Effects such as line-broadening with increasing concentration, line-narrowing with a rise in temperature, etc., have been documented in tyrocidin B and other peptides, and were suggested to arise from self-association.[14] We have previously shown that raising the temperature of [Pen^1]oxytocin in $[^2H_6]Me_2SO$ results in an abrupt line narrowing of the spectra above about 55 °C.[15] Recently, Higashijima et al.[16] have noticed changes in the chemical shifts of the amide protons as a function of concentration for Pro-Leu-Gly-NH_2, the tripeptide side chain of oxytocin, in Me_2SO solutions. We have done a series of dilution experiments with oxytocin and [Pen^1]oxytocin and examined the high resolution proton FT-NMR of these solutions to look for these effects. At concentrations down to 10^{-3} M we saw no significant changes in peptide amide

chemical shifts or coupling constants. However, at low concentrations (10^{-3} M) of oxytocin, the hydroxyl proton of tyrosine-2 appeared. The failure to observe this signal in earlier studies of oxytocin and related compounds has been attributed to rapid exchange of this proton with water impurities in Me_2SO.[17,18] The amino terminal group of the half-cystine-1 was also believed to be involved in intramolecular catalysis of this exchange, since usually the tyrosine-2 hydroxyl resonance is observed in deaminooxytocin and analogues. However, our observation that at low concentrations of oxytocin in $[^2H_6]$-Me_2SO this hydrogen becomes observable, strongly indicates that at higher concentrations the catalytic effect which produces the rapid exchange of this proton with water has primarily an intermolecular rather than intramolecular origin, and offers perhaps the strongest evidence for an intermolecular interaction or association of oxytocin in this solvent. In this regard, it is interesting that even in deaminooxytocin the presence of the tyrosine-2 hydroxyl group is concentration dependent since at 5×10^{-2} M it was present,[17] but a 7×10^{-2} M[19] it was nearly absent. Indeed, examination of the literature[15,17-24] provides further indications of this phenomena for related analogues and derivatives of neurohypophyseal peptides. On the other hand, it appears that the formation of a complex of oxytocin with Ni^{2+} competes with this effect since addition of Ni^{2+} to an oxytocin solution in Me_2DMSO renders the tyrosine-2 hydroxyl hydrogen observable.[20]

We also conducted dilution experiments with $[^2H_6]Me_2SO$ solutions of [Pen^1]oxytocin, but even at 8×10^{-4} M, the hydroxyl proton of tyrosine-2 did not appear suggesting that even at this concentration, intermolecular interactions or association are important for this compound as was also suggested by the broad lines and featureless nature of the spectrum[14] even at concentrations of 1-5 mg/ml.

The results from the viscosity, carbon-13 T_1, and proton FT-NMR reported here suggest that some form of intermolecular interaction or association obtains for oxytocin in Me_2SO at high concentrations (>10 mg/ml) and for [Pen^1]-oxytocin throughout the concentration range examined. In the FT-NMR studies no significant changes in chemical shift or coupling constants for peptide backbone (NH and αCH) protons were observed, suggesting that whatever the interaction it does not cause a significant backbone conformational perturbation. No evidence was obtained to suggest that such an interaction occurs in aqueous solution. On the other hand, the carbon-13 T_1 results in Me_2SO solution imply that any interpretation of these data will be dependent on the experimental conditions chosen, and indicate that caution should be taken in interpretation of T_1 studies in concentrated organic solutions.

Acknowledgments. This work was supported by the National Science Foundation GB-40106 and PCM73-01197A01, and the U.S. Public Health Service AM-17420. We thank Drs. Frank Bovey and Elkan Blout for time on Bruker HX-270 spectrometers available to them.

References and Notes

(1) V. J. Hruby in "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins", Vol. 3, B. Weinstein, Ed., Marcel Dekker, New York, N.Y., 1974, pp 1-188.
(2) R. Deslauriers and I. C. P. Smith, *Top Carbon-13 NMR Spectrosc.* in press.
(3) J. D. Glickson in "Peptides: Chemistry, Structure and Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, pp 787-802.
(4) W. Y. Chan, R. Fear, and V. Du Vigneaud, *J. Pharmacol. Exp. Ther.*, **190**, 77 (1974).
(5) V. J. Hruby, F. Muscio, C. M. Groginsky, P. M. Gitu, D. Saba, and W. Y. Chan, *J. Med. Chem.*, **16**, 624 (1973); D. A. Upson and V. J. Hruby, *J. Org. Chem.*, **41**, 1353 (1976).
(6) A. F. Spatola, D. A. Cornelius, V. J. Hruby, and A. T. Blomquist, *J. Org. Chem.*, **39**, 2207 (1974).
(7) M. Blumenstein and V. J. Hruby, unpublished results.
(8) J. H. Bradbury in "Physical Principles and Techniques of Protein Chemistry", Part B, S. Leach, Ed., Academic Press, New York, N.Y., 1970, pp 99-145.
(9) R. Deslauriers, I. C. P. Smith, and R. Walter, *J. Am. Chem. Soc.*, **96**, 2289 (1974).
(10) R. Walter, I. C. P. Smith, and R. Deslauriers, *Biochem. Biophys. Res. Commun.*, **58**, 216 (1974).
(11) A. Abragam, "The Principles of Nuclear Magnetism", Oxford University Press, London, 1967, Chapter VIII.
(12) I. Solomon, *Phys. Rev.*, **99**, 559 (1955).
(13) A. L. McLaughlin and J. S. Leigh, Jr., *J. Magn. Reson.*, **9**, 296 (1973).
(14) L. C. Craig, W. A. Gibbons, and M. Printz in "Progress in Peptide Research", Vol. II, S. Lande, Ed., Gordon and Breach Science Publishing, New York, N.Y., 1972, pp 217-233.
(15) J.-P. Meraldi, D. Yamamoto, V. J. Hruby, and A. I. R. Brewster in "Peptides: Chemistry, Biology and Structure", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, pp 803-814.
(16) T. Higashijima, N. Tasumi, and T. Miyazawa, *FEBS Lett.*, **57**, 175 (1975).
(17) L. F. Johnson, I. L. Schwartz, and R. Walter, *Proc. Natl. Acad. Sci. U.S.A.*, **64**, 1269 (1969).
(18) J. D. Glickson, D. W. Urry, R. T. Havran, and R. Walter, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2136 (1972).
(19) A. I. R. Brewster, V. J. Hruby, J. A. Glasel, and A. E. Tonelli, *Biochemistry*, **12**, 5294 (1973).
(20) R. Walter, R. T. Havran, I. L. Schwartz, and L. F. Johnson in "Peptides, 1969", E. Scoffone, Ed., North-Holland Publishing, Amsterdam, 1971, pp 255-265.
(21) P. H. von Dreele, A. I. Brewster, H. A. Scheraga, M. F. Ferger, and V. du Vigneaud, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1028 (1971).
(22) A. I. Brewster, J. A. Glasel, and V. J. Hruby, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1470 (1972).
(23) R. Walter, J. D. Glickson, I. L. Schwartz, R. T. Havran, J. Meienhofer, and D. W. Urry, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1920 (1972).
(24) R. Walter, A. Ballardin, I. L. Schwartz, W. A. Gibbons, and H. R. Wyssbrod, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 4528 (1974).

Jean-Paul Meraldi, Victor J. Hruby*
Department of Chemistry, University of Arizona
Tucson, Arizona 85721
Received March 24, 1976

5,8,16,19-Tetra-*tert*-butyl-6,17,23-trisdehydro[22]-bi[10.10.2]annulene.[1] A Condensed Nonbenzenoid Aromatic System Consisting of Two 14π-Electron Systems

Sir:

In a previous paper,[2] we have reported the synthesis of a condensed nonbenzenoid aromatic hydrocarbon (XIII), which can be regarded as a fused system of two tetrakisdehydro[18]-annulene derivatives. At the same time, the synthesis of an ortho-fused 26π-electron [14]annuleno[14]annulene derivative was reported by Cresp and Sondheimer.[3] In this communication, we wish to report the synthesis and properties of the third instance of a fused nonbenzenoid aromatic hydrocarbon (XII), a lower analogue of XIII consisting of two 14π-electron systems.

Treatment of the ethynyl ketone (III: yellow crystals; mp 104.5-105.0 °C; 83%; Anal. ($C_{17}H_{24}OS$) C, H, S),[4] obtained by the aldol condensation of II[5] with I[6] (NaOH-H_2O-EtOH, 0 °C, 4 h), with Et_2NLi in THF at −78 °C followed by the reaction with Me_3SiCl gave trimethylsilyl derivative (IV: yellow liquid; 78%). Product (V) of the reaction of IV with lithium acetylide in THF[7] (−65 °C, 1 h; −55 °C, 1.5 h) was treated without isolation with 2 N H_2SO_4 (30 °C, 1 h) to yield VI (orange yellow liquid; 72%; 2,4-dinitrophenylhydrazone: red crystals; mp 225-227 °C; Anal. ($C_{24}H_{28}N_4O_4Si$) C, H, N). VI was converted into dimethyl acetal (VII) in the usual way, and treated with BuLi in THF (−78 °C) to give lithio derivative (VIII). The reaction of VIII with IV (−65 °C, 1 h; −45 °C, 1 h) followed by rearrangement and hydrolysis with 2 N H_2SO_4 (30 °C, 0.5 h) yielded dialdehyde (IX: yellow crystals; mp 142-143 °C dec; 39% based on IV; Anal. ($C_{34}H_{46}O_2Si_2$) C, H).[8] After several unsuccessful trials, conversion of IX into diketone (X: yellow crystals; mp 212-213 °C dec; 81%; Anal. ($C_{46}H_{66}O_2Si_2$) C, H) could be achieved