ceed via hydrolysis of **8a** by water associated with the oxidant or formed during the oxidation.

Although NiO_2 could not be employed for the conversion **8a** → **6a,** this reagent has also been used for the attempted oxidation of other partially reduced N-, O-, and S-containing heterocycles, many of which were dehydrogenated in good yield. Compounds oxidized successfully with NiO_2 included 2-methylthio-Δ^2-thiazoline (60%), methyl 2-methyl-Δ^2-imidazoline-4-carboxylate (81%), 1,5-diphenyl-3-(*p*-bromophenyl)pyrazoline (95%),[14] 2,3-dihydrobenzofuran (52%),[15] and several 2,4-disubstituted Δ^2-thiazolines, including phleomycin A_2 (83%).[16]

Acknowledgments. We thank Professor George Büchi and Dr. John Vederas for helpful discussions during the course of this work, Dr. Robert Engle (National Cancer Institute) for providing an authentic sample of 2′-(2-aminoethyl)-2,4′-bithiazole-4-carboxylic acid, and Dr. H. Umezawa for samples of phleomycin A_2 and bleomycin A_2. This investigation was supported by contract N01-CM-43712 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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(6) Compound **2a** (R′ = H) was obtained (100%) by successive treatments of *N*-acetyl-β-alanyl-*S*-tritylcysteine ethyl ester (**2a,** R′ = $(C_6H_5)_3C$) with $HgCl_2$ or $AgNO_3$ and then with methanolic H_2S. The fully blocked dipeptide was prepared by condensation of *N*-acetyl-β-alanine and *S*-tritylcysteine ethyl ester (79%; DCC, *N*-hydroxysuccinimide).

(7) E.g., (a) potassium ferricyanide and mercuric acetate, J. Walker, *J. Chem. Soc. C*, 1522 (1968), and N. A. Fuller and J. Walker, *ibid.*, 1526 (1968); (b) hydrogen peroxide and potassium dichromate, F. Asinger, M. Thiel, and L. Schroeder, *Justus Liebigs Ann. Chem.*, **610**, 49 (1957); (c) cupric sulfate;[5b] (d) MnO_2 and various quinones, M. A. Barton, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc. C*, 1061 (1966).

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(9) New compounds gave satisfactory elemental analyses or high resolution mass spectra.

(10) The two oxidants gave comparable yields for this transformation.

(11) Compound **6b,** mp 143–145 °C, λ_{max} (C_2H_5OH) 290 nm (log ε 4.18), has been prepared previously: K. Y. Zee-Cheng and C. C. Cheng, *J. Heterocycl. Chem.*, **7**, 1439 (1970).

(12) Obtained from **2a** (R′ = $(C_6H_5)_3C$) in 67% overall yield via saponification (methanolic NaOH, reflux, 10 min), condensation with *S*-tritylcysteine ethyl ester (DCC, *N*-hydroxysuccimmide) to give **7a** (R′ = $(C_6H_5)_3C$) and deblocking via the monomercuric mercaptide.

(13) Alternate workup procedures, which permitted the product to come in contact with an aqueous phase, gave material with much smaller apparent absorptivitiy. Although ε is dependent on the nature of the ring substituents and the medium in which the UV spectrum is recorded, molar absorptivity values of 5000 for single thiazolines are typical. See, e.g., (a) W. Stoffel and L. C. Craig, *J. Am. Chem. Soc.*, **83**, 145 (1961); (b) W. Konigsberg and L. C. Craig, *J. Am. Chem. Soc.*, **81**, 3452 (1959), and ref 7a.

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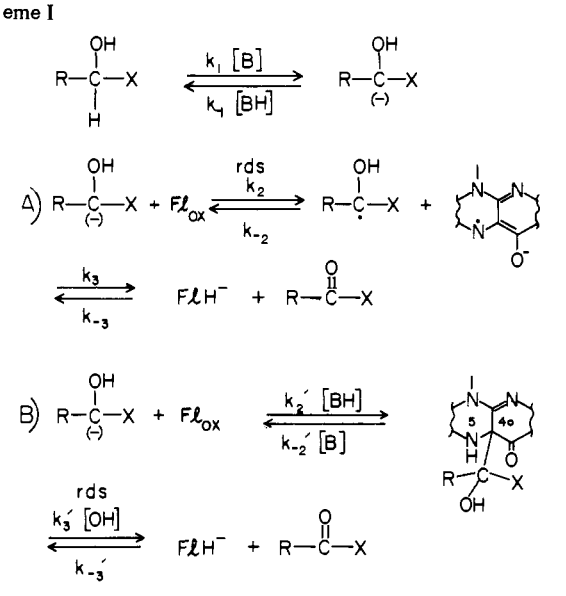
Oxidation of 9-Hydroxy- and 9-Methoxyfluorene Carbanions by Flavin. Proof of Radical Mechanism

Sir:

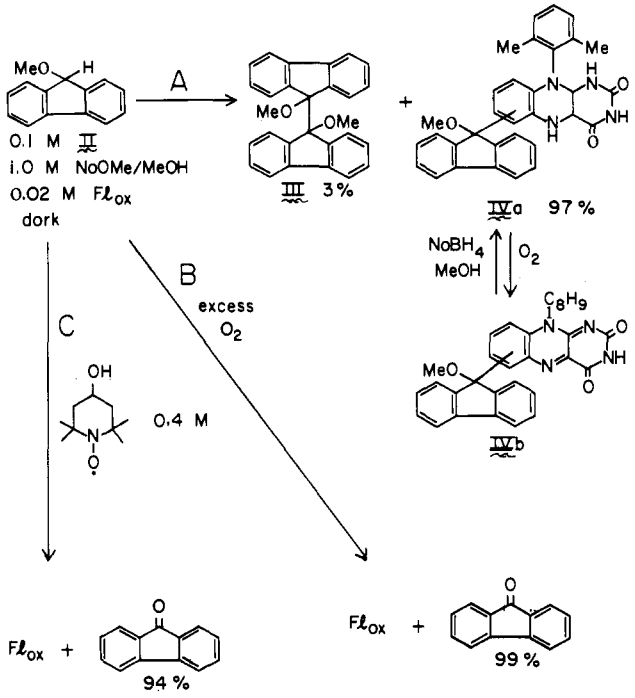
Flavin mediated dehydrogenation reactions which introduce unsaturation α,β to carbonyl groups are of considerable biochemical interest (lactic acid oxidase, amino acid oxidases, succinic acid dehydrogenase, etc.) and have been the subject of numerous investigations.[1-3] Model studies from this laboratory[2b,3b,d] have firmly established that it is the resonance stabilized carbanion of the substrate which undergoes oxidation by flavin. Kinetic and other evidence supports a radical mechanism (Scheme IA) or, less likely, a mechanism involving a 4a adduct which goes on to product by specific base catalysis (Scheme IB).[2b,3,4]

The mechanism of Scheme IA has been favored[3] on the basis of free-energy calculations,[3c,e] arguments centered around the requirement of specific base catalysis of 4a-adduct decomposition,[3d] and the results of studies with 1,5-dihydro-3,5-dimethyllumiflavin.[5] However, direct evidence for the formation of a flavin–substrate radical pair, as required by Scheme IA, has not been obtained. The present study deals

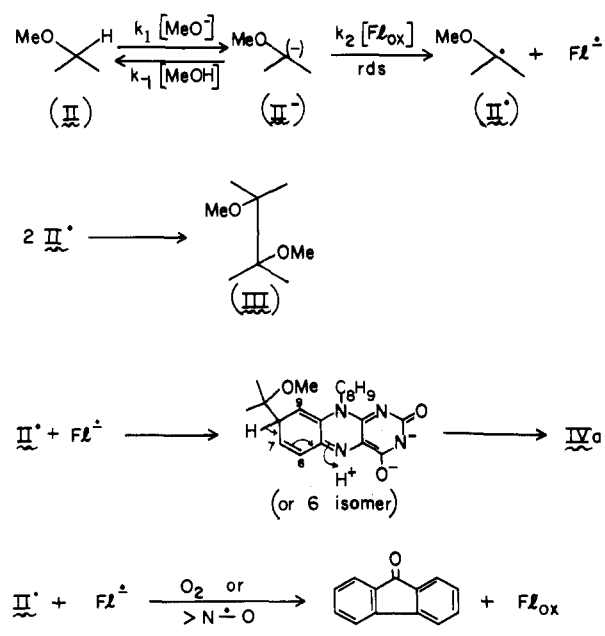
Scheme I



Scheme II



Scheme III



with the oxidation of the carbanions of 9-hydroxyfluorene (I^-) and 9-methoxyfluorene (II^-) in methanol solution by 10-(2′,6′-dimethylphenyl)isoalloxazine[6] (Fl_{ox}). If Scheme IA is correct, then I^- should yield fluorenone, while II^- should form 9-methoxyfluorene radical (II·) whose presence is expected to become evident through the appearance of radical coupling products.[7]

When Fl_{ox} (1×10^{-4} M) and either 9-hydroxyfluorene (I) or 9-methoxyfluorene (II) (0.10 M) are combined, in the dark, in basic methanol (1.0 M NaOMe, 30 °C) in the absence of O_2, the visible spectrum of Fl_{ox} disappears and is replaced by a spectrum characteristic of a reduced flavin. In the case of I, readmission of O_2 at the end of the reaction leads to restoration (>99%) of a spectrum characteristic of the original oxidized flavin in basic methanol (λ_{max} 441 nm, ϵ 8900 M^{-1} cm^{-1}). A product study under identical conditions, except for flavin concentration, confirmed the oxidation product to be fluorenone (isolated in 93% yield) as would be expected from the results of other studies.[1,3] The recovered reoxidized flavin was shown to be identical with the original flavin by NMR spectroscopy. In the case of II, readmission of O_2 at the end of the reaction regenerated an oxidized flavin, but it was obvious from its spectrum (λ_{max} 446 nm, $\epsilon \simeq 12\,000$ M^{-1} cm^{-1}) that it was not the starting Fl_{ox}. A series of product studies (Scheme II) were undertaken in the presence and absence of radical trapping agents which provided products whose formation is ascribable to radical coupling processes. Control experiments showed that the reactions of Scheme II did not occur in the absence of either Fl_{ox} or sodium methoxide.

The partial structure of the new flavin (IV) isolated from the reaction of II^- and Fl_{ox} under anaerobic conditions (Scheme IIA) was assigned on the basis of NMR, mass, and UV-vis spectral data.[8] In particular, the UV-vis spectra of IVa and IVb are inconsistent with either a 4a or 5 adduct[9,10] and the NMR spectrum of IVb is inconsistent with a 4a adduct since 4a adducts of 10-(2′,6′-dimethylphenyl)isoalloxazines are known to show splitting of the methyl absorptions of the 2′,6′-dimethylphenyl group in the NMR.[10] Products isolated from the alkaline and HI hydrolysis of IVb were consistent with the assigned structure. In particular, the reductive hydrolysis with HI indicated that the fluorene moiety is attached to the flavin at the 6 or 8 position.[11] The structure of III was confirmed by comparison with authentic dimer obtained by radical oxidation of 9-methoxyfluorene by nitrobenzene in basic methanol.[7]

In the presence of radical trapping agents (oxygen or 4-hydroxy-2,2,6,6-tetramethylpiperidinooxy[12] (>N-O·)) neither III nor IV could be detected as products; only fluorenone could be isolated. The mechanism of Scheme III best explains the results of the product studies of Scheme II.

The products formed under the conditions of Scheme IIB and IIC are those expected from the trapping of radical intermediates.[7,12a,13] It is well known that oxygen will trap flavin radicals to reform Fl_{ox},[13] and >N-O· has been shown to trap the radical form of the flavin moiety of glucose oxidase to give the oxidized enzyme.[12a] It is also known that oxygen reacts with II· in methanol to form fluorenone.[7] The mechanism by which II· is trapped by oxygen or >N-O· could involve either electron transfer or a peroxide or hydroxylamine intermediate.[14] The products formed in the absence of radical traps also indicate a free-radical mechanism. The radical formed from the reaction of II^- with Fl_{ox} cannot be oxidized to the ketone by dissociation of H^+ followed by $1e^-$ transfer or by H· transfer as apparently occurs in alcohol oxidation[3e,15] (Scheme IA); it must be trapped by Fl· or another 9-methoxyfluorene radical to form the flavin adduct IVa or the dimer III. In fact, the dimer III has previously been shown to be the major product of the free-radical oxidation of 9-methoxyfluorene by nitroaromatics in basic methanol.[7]

The products isolated in the presence and absence of radical trapping agents and the overall similarity of these reactions to the known free-radical oxidation of 9-methoxyfluorene in basic methanol by nitroaromatics[7] are overwhelming evidence for the free-radical nature of the reaction of 9-methoxyfluorene with Fl_{ox}. These results also lend credence to the mechanism of Scheme IA for the oxidation of alcohols such as 9-hydroxyfluorene by flavins.

The kinetics of the oxidation reactions were followed in degassed methanol by monitoring the disappearance of Fl_{ox} at 441 nm under pseudo-first-order conditions.[16] The disappearance of Fl_{ox} was observed to follow first-order kinetics under all conditions. If oxidation involves rate limiting $1e^-$ transfer from the carbanion to Fl_{ox}, then the rate law

$$\frac{-d[Fl_{ox}]}{dt} = k[S][NaOMe][Fl_{ox}] \qquad (1)$$

Table I. Values of k_1 and k_2/k_{-1}' for 9-Methoxyfluorene at 30 °C in Methanol

[NaOMe], M	k (av), M^{-2} s^{-1} [a]	k_1, M^{-1} s^{-1} [b]	k_2/k_{-1}', M^{-1}
0.26	1.91×10^{-3}	1.72×10^{-4}	11.1
0.51	2.59×10^{-3}	2.15×10^{-4}	12.0
1.03	3.98×10^{-3}	2.87×10^{-4}	13.9

[a] These are average values taken from two runs at different concentrations of II. See note 17. [b] Calculated from data in ref 18 and 19.

should be followed where [S] is the concentration of either I or II. In practice the observed value of k increased with increasing base concentration when S = I or II.[17] This behavior has been observed previously for the methoxide catalyzed formation of II^- monitored by hydrogen-deuterium exchange of II in methanol-*O*-*d*.[18] This variation of k with [NaOMe] is apparently due to an increase in solvent polarity with increasing concentration of the base. The observed kinetics are therefore consistent with the mechanisms of Schemes IA and III for 9-hydroxyfluorene and 9-methoxyfluorene, respectively, with k_2 being rate determining in both cases so that for either substrate

$$k = k_1k_2/k_{-1}[\mathrm{MeOH}] = k_1k_2/k_{-1}' \qquad (2)$$

Comparison of the initial rate constants for the reaction of 9-hydroxyfluorene and 9-hydroxyfluorene-*9*-*d* with Fl_{ox} at [NaOMe] = 0.26 and 0.51 M provided $k_H/k_D = 4.7 \pm 0.2$. Since k_2/k_{-1}' is independent of the isotope of hydrogen at the 9 position it follows that $k_1^H/k_1^D \simeq 4.7$. This result confirms that the carbanion of I is the active species in the oxidation reaction and is consistent with the mechanism of Scheme IA.

For II, values of k_1 can be determined from the known rates of sodium methoxide catalyzed hydrogen-deuterium exchange of this compound in methanol-*O*-*d* at 30 °C,[18] and the solvent isotope effect, k_{MeOD}/k_{MeOH}, for ionization of the 9 hydrogen which is 2.4.[19] Table I lists the average values of k at each concentration of base, as well as the values of k_1, and the calculated ratios k_2/k_{-1}'. The data in Table I show that at a flavin concentration of 1×10^{-4} M the ratio $k_2[Fl_{ox}]/k_{-1}'$ is $\sim 1 \times 10^{-3}$. This value is consistent with the requirement that $k_2[Fl_{ox}] \ll k_{-1}'$ for Scheme III to give first-order kinetics in $[Fl_{ox}]$. The internal consistency of the data is further evidence in favor of the mechanism of Scheme III.

The pK_a of both I and II in methanol can be estimated to be 22.[20] With this value, the pK_a of methanol itself (18.3),[21] and the values of k_1 and k_2/k_{-1}' from Table I, it is possible to calculate approximate values for both k_2 and k_{-1}' for the oxidation of II.[22] At 1.03 M in sodium methoxide this calculation gives values for k_2 of 550 M^{-1} s^{-1} and k_{-1}' of 40 s^{-1}. The relatively small value of k_2 indicates that electron transfer from II^- to Fl_{ox} is not in the thermodynamically favored direction.[23] The magnitude of k_2 for the 9-hydroxyfluorene reaction (Scheme IA) is expected to be close to that of 9-methoxyfluorene owing to the similar configuration of the two carbanions and the similarity in substituent effects expected for the hydroxyl and methoxyl substituents. The near equivalence in the rate constants[17] for the oxidation of the two substrates by Fl_{ox} is, therefore, expected in view of their similar (if not identical) pK_a values in methanol if both reactions are proceeding via like mechanisms with electron transfer to Fl_{ox} (k_2) being the rate-determining step.

The demonstration of the radical nature of the oxidation of 9-methoxyfluorene by an isoalloxazine is the first direct evidence for the radical nature of carbanion oxidations by flavins. It is, therefore, an extremely important piece of evidence in terms of providing a rationale for deciding between the mechanisms of Scheme IA and IB for the oxidation of other carbanions.

Acknowledgment. This work was supported by grants from the National Science Foundation and The National Institutes of Health.

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(8) Identification was based on the following data for IVb: NMR (CD_2Cl_2) δ 8.45 (s, 1 NH), 8.2–6.7 (m, 14 H), 2.84 (s, 3 H), 1.90 (s, 6 H); mass spectrum (EI, 70 V) *m/e* 512, 317, 195; UV–vis (in methanol) λ_{max} 435 nm (ϵ 14 100 M^{-1} cm^{-1}), 348 (8030); UV–vis in 1.0 M sodium methoxide in methanol) λ_{max} 446 nm (ϵ 12 150 M^{-1} cm^{-1}), 340 (11 400). The UV–vis spectrum of IVa in 1.0 M sodium methoxide in methanol was typical of a reduced isoalloxazine: λ_{max} 360 (ϵ 6200 M^{-1} cm^{-1}). High resolution mass spectrum of IVb: *m/e* 512; calcd for $C_{32}H_{24}N_4O_3$, 512.185; obsd, 512.183. All data indicate that IV is not a mixture of adducts, but is a single product.
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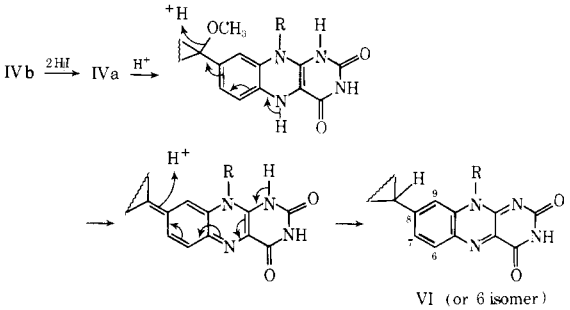

VI (or 6 isomer)

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(16) Kinetic conditions were as follows: methanol, 30 °C, $[Fl_{ox}] = 0.5–1.0 \times 10^{-4}$ M, [NaOMe] = 0.26–1.03 M, [II] = 0.05–0.10 M, or [I] = 0.0375–0.075 M. Fl_{ox} experienced no methanolysis on the time scale employed. No reaction was observed between Fl_{ox} and either substrate in the absence of base.
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(20) The pK_a of both I and II in Me_2SO can be estimated to be 16 from a correlation of pK_a values for five 9-substituted fluorenes in Me_2SO vs. σ_m ($\rho = -1.94$, $r = -0.98$). The pK_a data are from C. D. Ritchie, *J. Am. Chem. Soc.*, **91**, 6749 (1969), and C. D. Ritchie and R. E. Uschold, *ibid.*, **90**, 2821 (1968). The pK_a values in methanol can be estimated from the observation that $pK_a^{MeOH} - pK_a^{Me_2SO} = 6.0$ for several fluorene-type hydrocarbons. See the above references for the basis of this observation.
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(22) The values of k_{-1}' and k_2 are estimated as follows: the equilibrium between II and ^-OMe is

$$MeO^- + II \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} II^- + MeOH, \quad K = \frac{k_1}{k_{-1}}$$

where $k_{-1}' = k_{-1}[MeOH]$, but K is related to the pK_a of II and methanol by $pK = pK_a^{II} - pK_a^{MeOH}$. Using the values given in the text we calculate $pK \approx 3.7$. Then k_{-1}' can be calculated from $pK = \log k_{-1}' - \log k_1 - \log [MeOH]$. At 30 °C [MeOH] = 24.3 M, and values of k_1 are available from the data in Table I. The value of k_2 can then be obtained from the ratios k_2/k_{-1}' in Table I.

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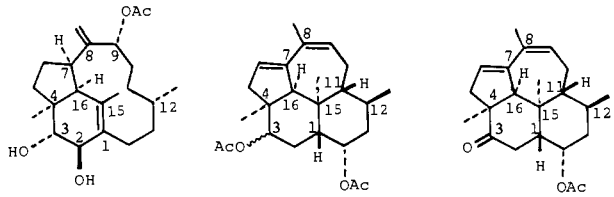
Received September 6, 1977

Kempene-1 and -2, Unusual Tetracyclic Diterpenes from *Nasutitermes* Termite Soldiers

Sir:

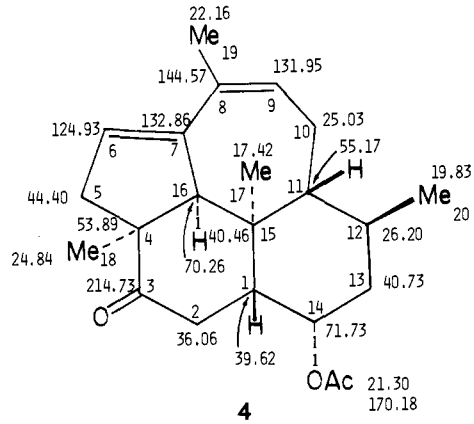
The glue-like secretions of nasute termite soldiers play a crucial role in the defense of these termite species against predacious ants.[1] Recently, we have described the structures[2,3] of the trinervitenes, e.g., **1** (TG-2), and the chemical composition of the secretion of the termite *Trinervitermes gratiosus* from which these compounds were first identified.[4] In this communication we reveal the structures of two new diterpenes, kempene-1 and -2, possessing novel tetracyclic cembrene-derived carbon skeletons.[5]



1 (TG-2) 2 (NK-1) 3 (NK-2)

Kempene-1 (or NK-1, **2**) and kempene-2 (or NK-2, **3**) were isolated from the hexane extract of crushed heads of *Nasutitermes kempae* soldiers by chromatography over Florisil followed by HPLC on μ-Porasil.[6] Kempene-2 was readily oxidized on standing under ambient conditions to give a substance containing one extra oxygen, a behavior which led to some confusion in the initial analysis of the spectral data.[7] The physical constants of **3** are as follows: mp 120.5–122.5 °C; mass spectrum *m/e* 342 (M^+) ($C_{22}H_{30}O_3$), 282 (M^+ − AcOH), 267 (M^+ − AcOH − Me); UV (MeOH) 244 nm (ε 6330); CD (MeOH), 241 (Δε +0.025, diene), 289 nm (Δε +1.46, ketone); IR (CCl_4) 1737 (OAc), 1702 cm^{-1} (ketone). The nature of all 22 carbons was determined (see **4**, Figure 1) by the ^{13}C NMR techniques of PND, selective decoupling, partially relaxed Fourier transform (PRFT), and combined PRFT/selective decoupling.[8]

The complex ^1H NMR spectrum (see **5**, Figure 1) was analyzed in detail at 100 MHz and 220 MHz. With the aid of $Eu(fod)_3$, it was possible to separate, at least in part, practically all proton signals (see **5**). Decoupling of the separate peaks, in conjunction with correlation of proton signals to carbon signals by selective heteronuclear decoupling, clarified most of the molecular structure (indicated by thick lines in **5**) except for the linkages between C-1 and C-15 and between C-12 and C-13 and those extending from C-11.[8] The chemical shifts of some overlapping peaks shown by approximate values in **5** were estimated by comparisons with the $Eu(fod)_3$-shifted spectrum. Assignments of ^1H NMR signals were also aided by comparisons with the signals of kempene-1. The ddd shape (each *J* being 3 Hz) of the 14-H peak defines the axial nature of OAc



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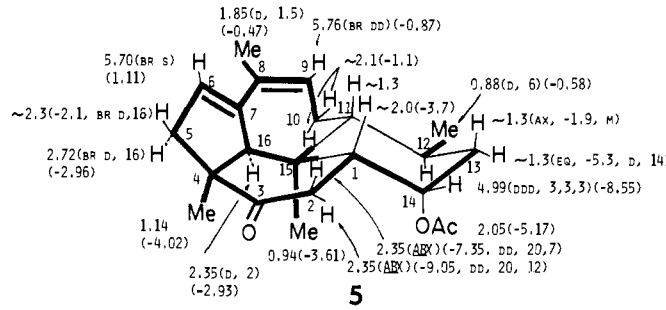

5

Figure 1. ^{13}C NMR data (**4**), JEOL PS-100 with microprobe, and ^1H NMR data (**5**), Varian HA-100 and HR-220, of NK-2 (**3**), both in $CDCl_3$. Negative ^1H NMR values shown in brackets in **5** denote downfield shifts occurring upon addition of $Eu(fod)_3$.

as well as the spatial relation between 14-H and the three adjacent protons. The large J_{gem} of the 2-H's (20 Hz) indicate the methylene to be adjacent to the ketone. The doublet nature (J = 2 Hz) of the isolated 16-H shows that it is long range coupled, probably to 5α-H, through overlap of the 5-H and 16-h σ bonds with the 6-ene π bond.

Kempene-1 (**2**) had mass spectrum *m/e* 386 (M^+) ($C_{24}H_{34}O_4$); UV (MeOH) 245 nm (ε 85);[9] CD (MeOH), 245 (Δε +0.058); IR ($CHCl_3$) 1730 cm^{-1} (OAc). The ^{13}C NMR resonances for C-2 and C-4 of kempene-2 (**4**, 36.06 and 53.89 ppm) are shifted to the high fields of 28.75 and 44.96 ppm, respectively. In the ^1H NMR spectrum, an additional carbinyl acetate proton (3-H) appears at 5.08 ppm (br d, J = 9 Hz), and hence the 3-one is replaced by a 3-acetate. Reduction of 4 mg of kempene-2 (**3**) with $LiAlH_4$ followed by acetylation and HPLC separation (μ-Porasil, 12% ether in hexane) afforded an ~1:1 mixture of **2** and its C-3 epimer: UV (MeOH) 243 nm (ε 85);[9] CD (MeOH) 244 nm (Δε +0.069); ^1H NMR ($CDCl_3$) 5.17 ppm (br d, J = 8 Hz, 3-H). The 3-H signal of **2** and 3-epi-**2** merely indicated that in both compounds the 3-acetoxyl group is equatorially oriented (owing to conformational inversion), and hence it was not possible to assign configurations to this center either by NMR or other evidence.

The structure of **3** was solved by single-crystal x-ray diffraction experiments. Kempene-2 crystallized from olefin-free pentane under an argon atmosphere in the orthorhombic crystal class. Cell constants, determined by least-squares fitting of 15 high angle reflections, were a = 10.370 (3), b = 9.671 (2), and c = 19.817 (7) Å. Systematic extinctions combined with the chirality of **3** indicated space group $P2_12_12_1$ with one molecule of composition $C_{22}H_{30}O_3$ per asymmetric unit (ρ = 1.14 g cm^{-3}). All unique data with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated Cu Kα (1.54178 Å) x rays. Of the 1596 diffraction maxima surveyed, 1258 (79%) were considered to be observed ($F_o^2 \geq 3\sigma(F_o^2)$) after correction for Lorentz, polarization, and background effects.

The angular dependence of the scattering was eliminated