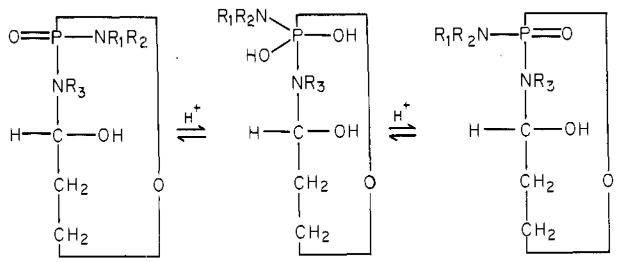
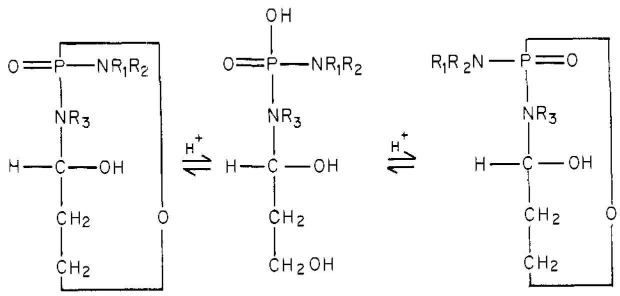
Scheme I



Scheme II



It is not difficult to envision a mechanism of action for the acid-catalyzed interconversion of *cis*- and *trans*-HPIPA (or the comparable hydroperoxy or hydroxy isomers of CPA) with the retention of configuration at C(4) (Scheme I). Protonation of the phosphoryl oxygen atom, followed by nucleophilic attack by a water molecule, would lead to a dihydroxylated, pentacoordinate configuration at the phosphorous. Either stereoisomer could then be obtained, depending on which of the chemically equivalent hydroxy groups is removed in the subsequent dehydration.

A second, perhaps less likely, pathway; somewhat analogous to the well-known mechanism for mutarotation of sugars, could occur via protonation of the ester oxygen, leading to a short-lived, ring-opened intermediate that would recyclize with resultant random configuration at the phosphorus but with no change at C(4) (Scheme II). This second mechanism provides an alternate pathway for the decomposition of 4-hydroxycyclophosphamide to acrolein and phosphoramide mustard other than through an aldophosphamide intermediate. If scission of the N(3)–C(4) bond occurs to this ring-opened form of cyclophosphamide (possibly via an aldolase enzyme), with coincident aldehyde formation, the products would be phosphoramide mustard and $CH_2(OH)CH_2CHO$. The latter would immediately undergo dehydration to form acrolein. It is also of interest to note that the ring-opened intermediate is the 4-hydroxy derivative of cytotoxyl alcohol and, thus, would be expected to be a potent cytotoxic agent itself.

Acknowledgment. We thank Dr. A. Takamizawa for supplying the crystals of HPIPA. This work was supported in part by the National Institutes of Health (Grant CA 15879) and by the Medical Research Council of Canada.

Registry No. *cis*-3, 64858-36-2; *trans*-3, 59884-18-3.

Supplementary Material Available: Table of fractional atomic coordinates and anisotropic thermal parameters and tables of observed and calculated structure factors (21 pages). Ordering information given on any current masthead page.

Structures of Two Isomeric Bicyclic Derivatives of 4-Hydroperoxyisophosphamide

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Crystal structure determinations of C4-oxygen-substituted cytotoxic derivatives of the anticancer drug cyclophosphamide have all found the oxygen to be in the axial position, suggesting an inherent stability for this geometry. Recently, two isomeric bicyclic derivatives of 4-hydroperoxyisophosphamide (cyclized *cis*- and *trans*-HPIPA) have been obtained for which NMR coupling constants imply that the trans isomer has the C4-oxygen substituent in the equatorial position. Crystal structure determinations of both bicyclic compounds have now been performed. They show that the cis isomer has phosphoryl oxygen and C4-peroxy group both axial, similar to the conformation of the uncyclized HPIPA precursor and to the expectation based on NMR data; the trans isomer, however, has a phosphoryl oxygen equatorial, C4-peroxy group axial conformation, similar to its uncyclized HPIPA precursor but opposite in conformation at both positions to the NMR-based inferences. The oxazaphosphorinane ring in each isomer has a half-chair conformation, with the trans isomer probably flipping between two equally probable half-chairs; this disorder may account for the observed differences in the NMR C4-hydrogen coupling constants in the two isomers. The peroxy-containing ring adopts a chair conformation in both molecules.

Cyclophosphamide (CPA) is one of the most widely used drugs in the treatment of many types of cancer. CPA itself has little cytotoxic activity in mammalian cell cultures; there is considerable evidence that in vivo activation proceeds via hydroxylation at C4 of the 1,3,2-oxazaphosphorinane ring. Either 4-hydroxycyclophosphamide or a further degradation product, phosphoramide mustard, is generally believed to be the ultimate cancerotoxic selective agent. In either case, the synthesis of preactivated analogues of CPA is desirable both for enhancement of activity and for an understanding of the pathways of CPA activation and metabolism.

Takamizawa synthesized 4-hydroxy- and 4-hydroperoxycyclophosphamide and found that both have cytostatic activity;[2] determination of the crystal structure of the latter compound[3] revealed the configuration at the phosphorus atom to be phosphoryl oxygen axial and bis(chloroethyl)amine group equatorial, as found in a number of cyclophosphamide analogues, and the configuration at C4

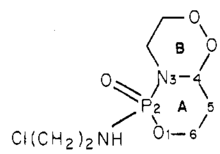
(1) (a) University of Toronto. (b) Present address: North Island College, Campbell River, B. C.. (c) University of Washington.

(2) Takamizawa, A.; Matsumoto, S.; Iwata, T.; Katagiri, K.; Tochino, Y.; Yamaguchi, K. *J. Am. Chem. Soc.* **1973**, *95*, 985–986. Takamizawa, A.; Matsumoto, S.; Iwata, T.; Katagiri, K.; Tochino, Y.; Yamaguchi, K.; Shiratori, O. *J. Med. Chem.* **1975**, *18*, 376–383.

(3) Camerman, A.; Smith, H. W.; Camerman, N. *Biochem. Biophys. Res. Commun.* **1975**, *65*, 828–832; *Acta Crystallogr., Sect. B* **1977**, *33*, 678–683.

to be hydroperoxy group axial. Similar configurations at these centers were found[4] in 4-peroxycyclophosphamide, a dimeric oxidation product of CPA with cytotoxic properties.[5] Two epimeric 4-hydroperoxy derivatives of the CPA analogue isophosphamide (IPA) were also prepared by Takamizawa, both exhibiting similar activities.[6] As described in the preceding article in this issue,[7] we determined the crystal structures of both isomers and found that they have opposite configurations at the phosphorus (phosphoryl oxygen axial in one and equatorial in the other), but both have axial hydroperoxy groups at C4. Therefore, it may be that an axial oxygen function at C4 is necessary for activity.[8]

Recently, Takamizawa has produced two bicyclic peroxides (I) formed by treating the epimeric 4-hydroperoxyisophosphamide (HPIPA's) with alkali, which produces linkage of the C4 peroxide to the N3 mustard substituent with loss of HCl. The bicyclic epimers are interconvertible in acid solution, exactly as are their HPIPA precursors. The relevant NMR coupling constants of one of these compounds was similar to those of the HPIPA's, and a phosphoryl oxygen axial, C4-peroxy group axial configuration, similar to its parent compound, was inferred.[6] The NMR data for the second bicyclic peroxide were significantly different, however, and a phosphoryl oxygen axial, C4-peroxy equatorial configuration, opposite at both loci to the parent compound, was proposed.[6]



I

If the structural assignments are correct, inversion of the configuration at C4 in the latter compound would be required during or after cyclization, and this would be the first example of a cyclophosphamide analogue with a C4 equatorial oxygen function. This result would seriously question conclusions drawn from the previous crystallographic works about the inherent stability and cytotoxic activating properties of a C4-oxygen axial conformation for the cyclophosphamide compounds. Accordingly, we undertook crystal and molecular structure determinations of both isomers in order to establish conclusively their stereochemistries at C4 and at phosphorus and to provide structural data that may aid in interpretation of the NMR spectra of these compounds.

Experimental Section

Crystals of both isomers were obtained by slow evaporation from 2-propanol/water solution. For working purposes, the isomer thought to have phosphoryl oxygen and C4-peroxy substituent both axial was called the cis isomer, and the other, assigned the phosphoryl oxygen axial, C4 peroxy equatorial configuration from the NMR data, was termed the trans isomer. Crystal data for both are given in Table I; unit cell parameters were obtained by diffractometer measurements of a number of axial, zonal, and general reflections.

Table I. Crystal Data for Cyclized Hydroperoxyisophosphamides

	cis isomer	trans isomer
formula	$C_7H_{14}N_2O_4ClP$	$C_7H_{14}N_2O_4ClP$
molar mass, g	256.63	256.63
crystal shape	needles along *a*	plates, elongated along *c*
crystal form	monoclinic	monoclinic
a, Å	6.665 (6)	16.839 (8)
b, Å	8.925 (40)[a]	6.883 (4)
c, Å	18.911 (14)	10.186 (4)
β, deg	98.45 (6)	102.14 (6)
U, Å^3	1113 (7)	1152 (2)
d_x g/cm^{-3}	1.53	1.48
Z	4	4
μ (Mo Kα), cm^{-1}	4.9	4.7
space group	$P2_1/c$	$P2_1/c$

[a] Average of values measured before and after data collection.

A. Cyclized *cis*-Hydroperoxyisophosphamide. The intensities of all reflections having 2θ (Mo $K\alpha$) < 50° (corresponding to a minimum interplanar spacing d = 0.84 Å) were measured on an automated four-circle diffractometer with zirconium-filtered Mo $K\alpha$ radiation using the 2θ–θ scan technique. Empirical absorption corrections, obtained by measuring the (200) reflection at χ (diffractometer) = 90° and at intervals of 30° in ϕ, were made. The variation in intensity was ±6%. The intensities were then corrected for background and linearly corrected for fall off in intensity (~25%), and Lorentz and polarization factors were applied. A total of 1936 unique reflections were measured, of which 1189 (61%) had $I > 2\sigma(I)$ and were considered to be observed.

Structure Determination. The structure was solved by routine application of the multiple-solution tangent formula program, MULTAN. Three cycles of isotropic least-squares refinement using statistical weights based on the coordinates of the heavy atoms from the E map gave R = 0.17, R_w = 0.13. One cycle of anisotropic least-squares refinement gave R = 0.15, R_w = 0.11, and the following difference Fourier map revealed the positions of all 14 hydrogen atoms. Four more cycles of refinement with the heavy atoms anisotropic and the hydrogen atoms isotropic gave final residuals of R = 0.115, R_w = 0.070 (for observed reflections only, R = 0.061, R_w = 0.059). Nineteen observed reflections having 50° < 2θ < 55° were included in the refinement, giving a total of 1955 reflections. Statistical weights were used throughout and the final $[\sum w\Delta^2/(m-n)]^{1/2}$ = 1.35. The maximum shift/error in the final cycle was 0.2 for the heavy atoms and 0.3 for the hydrogen parameters. Scattering factors utilized were those cited in the preceding paper. The atomic fractional coordinates, thermal parameters, and measured and calculated structure factors are available.[9]

B. Cyclized *trans*-Hydroperoxyisophosphamide. Data collection proceeded as for the cis isomer. A total of 1062 unique reflections were measured in the range 2θ (Mo $K\alpha$) < 40°; only 575 (54%) had $I > 2\sigma(I)$ and were classified as observed. The intensity crystal was small, and no absorption corrections were applied. Linear corrections for fall off in intensity (~25%) were made.

Structure Determination. The structure was solved by routine application of MULTAN. In order to obtain sufficient resolution of the E map, $|E|$ values greater than > 1.2 were used. Atoms C5 and C6 were poorly defined on the E map and have subsequently exhibited abnormally high thermal vibration in the crystallographic z direction. Three cycles of isotropic refinement starting with the coordinates from the E map gave R = 0.22 when all reflections were used and R = 0.14 when only the observed reflections were employed. The molecular parameters were more

(4) Sternglanz, H.; Einspahr, H. M.; Bugg, C. E. *J. Am. Chem. Soc.* **1974**, *96*, 4014–4015.
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(7) Camerman, A.; Smith, H. W.; Camerman, N. *J. Med. Chem.* **1983**, *26*, preceding paper in this issue.
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(9) See paragraph at the end of paper regarding supplemental material.

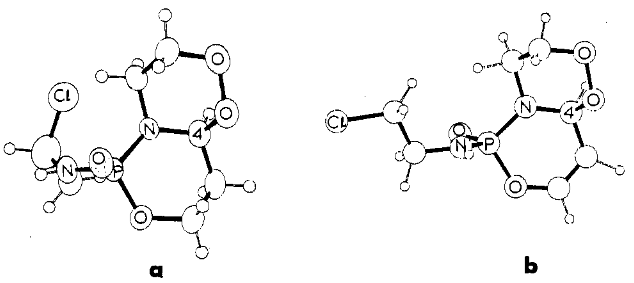

Figure 1. Perspective drawings of the crystal structures of (a) cyclized *cis*-HPIPA and (b) cyclized *trans*-HPIPA.

reasonable when the unobserved reflections were omitted, and therefore all subsequent refinement was based only on the observed reflections. Anisotropic refinement followed by difference Fourier maps enabled the location of 12 hydrogen atoms in calculated positions. Only one hydrogen attached to each of C5 and C6 could be found on the difference maps, in positions more closely corresponding to sp^2 bonding around C5 and C6 than to either of the calculated sp^3 positions. Anisotropic refinement of the heavy atoms with 12 hydrogen atoms included in the structure factor calculation (with the isotropic B's of the atoms to which they are bonded) but not refined gave a final $R = 0.066$. Unit weights were used throughout, and the final $[\sum w\Delta^2/m - n)]^{1/2} = 1.05$. The maximum shift/error in the final cycle was 0.2. A final difference Fourier synthesis showed a number of regions with $\rho = 0.2$–0.3 e/Å^3, some near O1 and C6, but despite the large thermal parameters of C5 and C6, there is no apparent evidence of statistical disorder. Scattering factors used were as previously cited. The fractional coordinates, atomic thermal parameters, and the observed and calculated structure factors are available.

Results and Discussion

A. Cyclized *cis*-Hydroperoxyisophosphamide. The molecular structure of cyclized *cis*-HPIPA is shown in Figure 1a. Unlike the uncyclized HPIPA precursor, the oxazaphosphorinane (or "A") ring is not in a chair conformation; rather, it is best described as a half-chair, with C6 lying 0.62 Å from the mean plane of the other ring atoms (maximum deviation of the others is 0.17 Å). The peroxy-containing (or "B") ring does adopt a chair conformation. As in *cis*-HPIPA, both the phosphoryl oxygen and the C4-oxygen atoms occupy axial positions with respect to the A ring and are on the same side of the ring and, hence, cis to each other. The O10–O11 separation is 3.81 Å, similar to the value of 3.76 Å in *cis*-HPIPA. The chloroethylamine group has a folded arrangement in the crystal.

Bond lengths and angles (and the atomic numbering scheme) are given in Figure 2. The values for cyclized *cis*-HPIPA are all near normal and require no special comment.

B. Cyclized *trans*-Hydroperoxyisophosphamide. The molecular structure of cyclized *trans*-HPIPA is shown in Figure 1b. As is the case with the cis isomer, the B ring has a chair conformation. The A ring appears roughly planar, but this undoubtedly is an artifact. The short distances and large interatomic angle involving C6 (Figure 2) are indicative, chemically, of an incorrect C6 atomic position. The most reasonable explanation would be that ring A actually adopts a half-chair conformation with C6 displaced from the mean plane of the other ring atoms, similar to the cis isomer, but that the C6 displacement is disordered among positions above and below the plane. This would explain the shortened bond lengths and large angle at C6 and would also account for the finding of only one hydrogen atom at each of C6 and C5, the observed positions corresponding to overlap of one hydrogen from each of the disordered conformations. As reported under Experimental Section, no apparent evidence for statistical

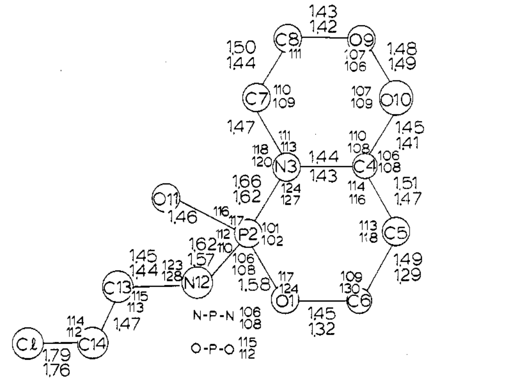

Figure 2. Bond lengths and angles in cyclized *cis*- (top numbers) and *trans*-HPIPA's.

disorder could be found from a difference electron-density calculation, but this is not surprising considering the rather poor quality of the crystallographic data for this compound.

Although the apparent planarity of ring A makes the distinction difficult, comparison of the structure of cyclized *trans*-HPIPA with the cis isomer (Figure 1) indicates that the conformation of the trans compound is best described as C4-oxygen axial, phosphoryl oxygen equatorial, similar to the situation in *trans*-HPIPA.[7] Thus, the nomenclature of trans for this isomer *does* describe the spatial relation of these two oxygen functions, but their configurations, in the crystal, are *opposite* to those proposed from the NMR data. The O10–O11 distance is 4.88 Å, again similar to the separation observed in *trans*-HPIPA (4.73 Å). The chloroethylamine chain is extended in this isomer.

The remainder of the bond lengths and angles (not involving C6) are similar to those in cyclized *cis*-HPIPA. There is one intermolecular hydrogen bond in each crystal structure, involving the chloroethylamine nitrogen and a phosphoryl oxygen of another molecule in both cases. The N12···O11 distances are 2.94 and 2.86 Å for the cyclized *cis*- and *trans*-HPIPA, respectively.

C. Correlation of Crystal Structures with NMR Spectra. The crystal structure determination of cyclized *cis*-HPIPA has confirmed the cis axial phosphoryl oxygen, axial C4-oxygen conformation expected from the structure of *cis*-HPIPA[7] and from the similarity of C4–H NMR coupling constants for the cyclized and uncyclized compounds. However, the cyclized *trans*-HPIPA molecular conformation in the crystal does not agree with the arrangement proposed on the basis of the NMR data. The markedly different C4–H coupling constants for this compound led Takamizawa et al. to infer that the configuation was phosphoryl oxygen axial, C4-oxygen equatorial, opposite at both centers to that of the precursor *trans*-HPIPA, whereas the crystal structure conformation is phosphoryl oxygen equatorial, C4-oxygen axial, similar to *trans*-HPIPA. We have recorded 220-MHz NMR spectra for cyclized *trans*-HPIPA and find that the data are consistent with the crystal structure results; i.e., the C4–H coupling constants are reasonable for a model in which the C4 hydrogen is equatorial and the C5 and C6 hydrogen atoms are statistically disordered by the very rapid interchange of two half-chair conformations for the oxazaphosphorinane ring, with C6 lying either above or below the plane of the other ring atoms. It is pertinent to note that although other possible configurations in solution cannot be ruled out by the NMR data, the C4-oxygen equatorial conformation postulated above[6] was predicated on a chair conformation for the oxazaphosphorinane ring, while the structure results presented here indicate that in both isomers cyclization causes deformation of this ring to a half-chair.

It is also of interest to note that since cyclized *cis*- and *trans*-HPIPA differ in configuration only at the phosphorus atom, interconversion could be mediated through the same mechanisms as proposed for uncyclized *cis*- and *trans*-HPIPA.[7]

D. Relationship of Conformation to Biological Activity. In forming the bicyclic peroxide from HPIPA, the chloroethyl group attached to N3 is inactivated by intramolecular reaction with the C4 hydroperoxide, leaving the cyclized HPIPA's with only one alkylating moiety. Thus, these compounds are not attractive as prospective antineoplastic agents, and their biological activity has not been investigated. Nevertheless, crystal structure determinations of the two cyclized HPIPA epimers has provided data highly relevant to structure–activity relationships in the cyclophosphamide family of anticancer drugs.

As stated earlier, all the C4-hydroxylated, preactivated, cyclophosphamide derivatives characterized to data have the C4-oxygen substituent in the axial position, regardless of whether hydroxylation has been achieved by ozonolysis of open-chain compounds[2,6] or by the Fenton oxidation of cyclophosphamide.[5] Since all of the synthetically prepared compounds are essentially equivalent in biological activity, it seemed reasonable to suggest[8] that this arrangement is the most stable one and is likely the configuration of the 4-hydroxy derivatives produced in the in vivo activation of cyclophosphamide and its analogues.

The validity of these conclusions was questioned, however, by the cyclized HPIPA NMR spectra, which were interpreted as showing the C4 oxygen in the equatorial position in the trans epimer. The change in conformation at C4 (and at phosphorus) in going from *trans*-HPIPA to cyclized *trans*-HPIPA was rationalized by postulating the C4-oxygen axial conformation to be an unstable intermediate.[6] If a change in the environment of the C4-oxygen atoms, such as in the formation of the bicyclic peroxide, can render the C4-oxygen axial geometry unstable and cause an inversion to the equatorial oxygen configuration, it is conceivable that similar forces could operate in the enzymatic hydroxylation process or during the cellular uptake of the hydroxylated derivatives. Crystal structure determinations of the cyclized HPIPA's demonstrate, however, that the C4-oxygen axial configuration is a stable arrangement for both epimers in the solid state and, furthermore, provide a stereochemical basis for reinterpretation of the cyclized *trans*-HPIPA NMR spectrum to suggest that this configuration is the stable one in solution also. Thus, these crystal structure results are consistent with and strongly reinforce the structure–activity correlations previously postulated for the cyclophosphamide family of drugs.

Acknowledgment. We thank Dr. A. Takamizawa for supplying samples of the title compounds and Drs. A. Grey and N. Andersen for helpful discussions of the NMR spectra. Support was from the Medical Research Council of Canada and from the National Cancer Institute, United States Public Health Service (Grant CA 15879).

Registry No. cyclized *cis*-HPIPA, 64858-46-4; cyclized *trans*-HPIPA, 64858-45-3.

Supplementary Material Available: Atomic coordinates, thermal parameters, and observed and calculated structure factors for both structures (17 pages). Ordering information is given on any current masthead page.

Quantitative Structure–Activity Relationship of Double Alkyl Chain Drugs

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The quantitative structure–activity relationship of double alkyl chain drugs, including alkanols, aliphatic esters, ketones, barbiturates, amphetamines, butyrylcholinesterase inhibitors, antimalarials, and rifamycin amides, is investigated. A series of double-chain homologues, $C_nH_{2n+1}XC_mH_{2m+1}$, in which n changes, keeping m constant, is classified into three types: in type IIL, $n > m$; in type IIE, $n = m$; in type IIS, $n < m$. When a linear relationship, vis., $\log (1/C) = an + b$, holds, the slope a depends on the type; $a_{\mathrm{I}} \geq a_{\mathrm{IIL}} > a_{\mathrm{IIE}} > a_{\mathrm{IIS}}$. Here a_{I} means the slope for single-chain homologues. The same order is observed for the equation, log hydrophobicity = $an + b$, where the hydrophobicity of drug denotes the water solubility, the critical micelle concentration, and the partition coefficient for the 1-octanol–water phases. Therefore, decreased biological activity of a double-chain drug relative to that of a single-chain isomer can be explained by a decreased hydrophobicity of the double-chain drug, due to the intramolecular association of these chains in water. When a parabolic relationship between $\log (1/C)$ and n holds, the optimum n depends on the type: $n_{\mathrm{opI}} < n_{\mathrm{opIIL}} < n_{\mathrm{opIIE}}$. This order is also explicable on the basis of a decreased hydrophobicity of double-chain drug. The N-dealklation rate of amphetamines in vivo appears to be affected by the steric factor as well as the hydrophobic factor. A decreased hydrophobicity of double-chain compounds should be taken into consideration for estimating their partition coefficients.

Hydrophobic substances or groups play an important role in forming the high-order structure of biomembranes, proteins, micelles, liposomes, etc. in aqueous media.[1] We have shown that such a hydrophobic effect exists in low-molecular-weight compounds; e.g., two or three alkyl chains of sulfoxides,[2] ethyleneglycol diesters,[3] and triglycerides[4] aggregate intramolecularly in aqueous solutions. The logarithms of the critical micelle concentration (cmc) of dialkyl sulfoxides and of the solubility (C_s) of ethylene glycol diesters and triglycerides in water are correlated linearly with the total number of carbon atoms (n) in these molecules according to eq 1, and the coefficients (a) for

$$\log (\text{cmc or } C_s) = -an + b \qquad (1)$$

these double- and triple-chain compounds are smaller than that for single-chain compounds.[2-4] A similar effect may

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