Membrane-Disrupting Surfactants That Are Highly Selective toward Lipid Bilayers of Varying Cholesterol Content[1]

Yoshinobu Nagawa[2] and Steven L. Regen*

Contribution from the Department of Chemistry and Zettlemoyer Center for Surface Studies, Lehigh University, Bethlehem, Pennsylvania 18015. *Received October 9, 1990*

Abstract: Membrane-disrupting bolaphiles I [$HO(CH_2CH_2O)_6CO(CH_2)_{14}CO_2(CH_2CH_2O)_6H$] and III [$HO(CH_2CH_2O)_6CO(CH_2)_6CH{=}CH(CH_2)_6CO_2(CH_2CH_2O)_6H$], and their polymeric counterparts II ($-[CO(CH_2)_{14}CO_2(CH_2CH_2O)_{13}]_{4.8}-$) and IV ($-[CO(CH_2)_6CH{=}CH(CH_2)_6CO_2(CH_2CH_2O)_{13}]_{5.7}-$), show high selectivity toward lipid bilayers of varying cholesterol content. In the absence of cholesterol, these surfactants are effective in inducing the release of 5(6)-carboxyfluorescein, entrapped within liposomes made from both egg PC and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). The ability of I-IV to disrupt POPC bilayers that contain substantial amounts of cholesterol (>33 mol %), however, is significantly reduced. In contrast, Triton X-100 and a single-chain analogue of II (i.e., $CH_3(CH_2)_6CO_2(CH_2CH_2O)_6H$, V) are relatively insensitive to the presence of cholesterol. Similar selectivity has been observed with biological targets, i.e., human erythrocytes and a human bacterium (*Proteus mirabilis*). These results provide the first clear evidence that modest and definable differences in membrane composition and packing can lead to large differences in lability, and that synthetic agents can be created which exploit such differences. The implications of these findings to the development of membrane-disrupting antimicrobial agents are briefly discussed.

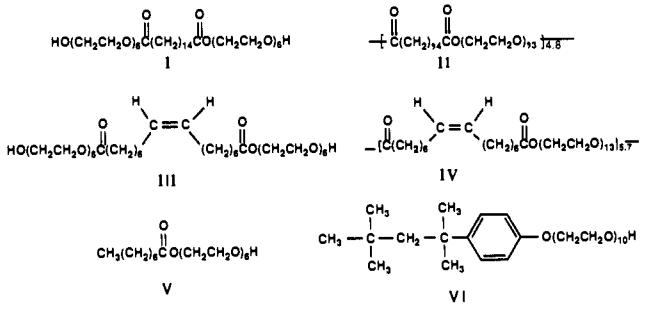
Introduction

Conceptually, the selective disruption of the lipid envelope of a microorganism in the presence of mammalian cells represents an intriguing strategy for the creation of novel antimicrobial agents.[3] One well-documented example of a membrane-disrupting compound that has assumed major clinical importance is Amphotericin B (Amp B). This macrolide antibiotic owes both its antimicrobial activity and its mammalian cell toxicity to its ability to perturb sterol-containing membranes. In particular, it has been shown that Amp B generates transmembrane pores that allow the leakage of vital cellular constituents, and trigger cell destruction.[4-8] Although Amp B shows only a modest degree of cellular selectivity, it has become an important agent for the treatment of fungal infections.

Despite growing interest in membrane-disrupting compounds as chemotherapeutic agents, the notion that such species can be tailor-made to selectively perturb a biological membrane is largely without experimental basis.[9,10] Moreover, those structural and/or compositional features of biomembranes that may be exploitable by a disruptive agent remain to be identified. Model studies are needed in order to clarify these issues and to help guide the rational design of new and more efficacious agents.

We have recently shown that double-headed, single-chain surfactants (bolaphiles) and their polymeric analogues (supramolecular surfactants) exhibit a wide range of activity in disrupting bilayers derived from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC).[11-13] We have also shown that the activity of such surfactants is very sensitive to the size of the hydrophobic segment, and to the presence of unsaturation. A model that was used to account for bolaphile structure-activity relationships assumes that the aliphatic segments insert into the outer monolayer leaflet as hydrocarbon loops, thereby generating defects. While the correctness of this model remains to be established, it does lead to a tantalizing prediction regarding their possible selectivity features. Unlike conventional detergents, which can readily "slither" into a lamellar phase as single chains, bolaphiles or supramolecular surfactants can only insert themselves into a membrane in the form of loops. On the basis of steric requirements, loops should have significant difficulty in penetrating tightly packed monolayers because they require the simultaneous insertion of two alkyl chains. Since cholesterol increases the compactness of fluid bilayers, it is reasonable to expect, therefore, that the disrupting power of a bolaphile and a supramolecular surfactant could be particularly sensitive to the cholesterol content of a lipid membrane (Figure 1).

With these ideas in mind, we have examined the ability of representative bolaphiles I and III, and their polymeric counterparts II and IV, to discriminate among a series of phosphatidylcholine membranes of varying cholesterol content. We have also compared their selectivity features with those of a single-chain analogue, V, and also the commonly used membrane-disrupting detergent Triton X-100 (VI). The primary objective of this study



was 2-fold: (i) to establish whether or not a membrane-disrupting surfactant could discriminate, effectively, among lipid bilayers of varying cholesterol content, and (ii) to determine whether or not a bolaphile and/or a supramolecular surfactant could provide significantly greater membrane-disrupting selectivity as compared with conventional single-chain analogues.

The specific targets that we have chosen for these experiments were 1000-Å-diameter unilamellar vesicles derived from (i) egg PC, (ii) POPC, and (iii) three different mixtures of POPC/cholesterol. The unsaturated lipids POPC and egg PC have low gel to liquid-crystalline phase transition temperatures (T_m) and are convenient to study in their physiologically relevant fluid phase.[14,15] The fact that egg PC is a complex mixture of natural

(1) Supported by PHS Grant A128220 awarded by the National Institute of Allergy and Infectious Diseases.
(2) On leave from the National Chemical Laboratory for Industry, Tsukuba, Japan.
(3) Reimund, E. *Lancet* **1986**, 1159.
(4) Edwards, J. E.; Lehrer, R. I.; Stiehm, E. R.; Rischer, T. J.; Young, L. S. *Ann Intern. Med.* **1978**, *89*, 91.
(5) Horn, R.; Wong, B.; Kiehn, T. E.; Armstrong, D. *Rev. Infect. Dis.* **1985**, *7*, 646.
(6) Hermans, P. E.; Keys, T. F. *Mayo Clin. Proc.* **1983**, *58*, 223.
(7) Miller, J. M. *Bacteriol. Rev.* **1973**, *37* (2), 166.
(8) Andreoli, T. E.; Monahan, R. *J. Gen. Physiol.* **1968**, *52*, 300.
(9) Snipes, W.; Person, S.; Keith, A.; Cupp, J. *Science* **1975**, *188*, 64.
(10) Aloia, R. C.; Jensen, F. C.; Curtain, C. C.; Mobley, P. W.; Gordon, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 900.
(11) Regen, S. L.; Jayasuriya, N.; Fabianowski, W. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 566.
(12) Jayasuriya, N.; Bosak, S.; Regen, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5844.
(13) Jayasuriya, N.; Bosak, S.; Regen, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5851.

0002-7863/91/1513-7237$02.50/0

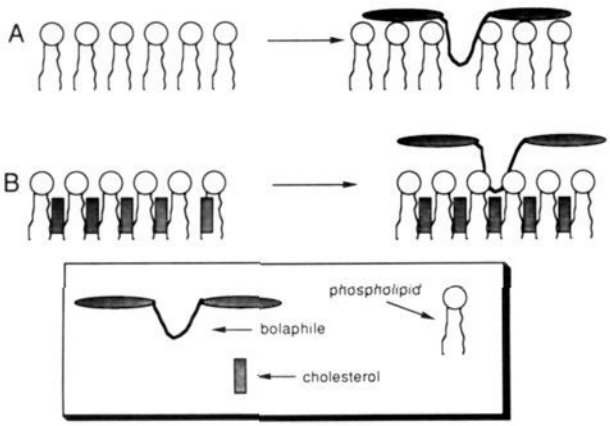


Figure 1. Stylized illustration of (A) a bolaphile inserting into and disrupting a fluid phospholipid membrane and (B) a bolaphile failing to insert into a cholesterol-rich membrane.

phosphatidylcholines, having saturated fatty acids largely in the sn-1-position and unsaturated acids in the sn-2-position, makes the synthetic analogue POPC more attractive, in terms of yielding better chemically defined bilayers. Egg PC based bilayers are, nonetheless, of interest because of their natural origin. In order to judge the disrupting properties of each surfactant, we have measured their ability to induce the release of 5(6)-carboxyfluorescein (CF), entrapped within liposomal compartments.[12,13,16,17]

Experimental Section

General Methods. Unless stated otherwise, procedures used throughout this work were similar to those which have been previously described.[12,13] The bacterium *Proteus mirabilis* (an L-phase variant) was purchased from the American Type Culture Collection (Rockville, MD) and was grown in heart infusion broth (DIFCO 0038, pH 7.5) with 20% inactivated horse serum and 10% (v/v) of a 25% (w/v) solution of fresh baker's yeast extract (GIBCO 360-8180). Critical micelle concentrations were determined in 10 mM borate buffer (pH 7.4, 140 mM NaCl, 2 mM NaN_3) by surface tension measurements using a Nima Model ST tensiometer (Coventry, U.K.).

Membrane-Disrupting Surfactants. Surfactants I–IV were prepared by using procedures similar to those which have been reported previously.[11,12] Analysis of the polymeric surfactants, by gel permeation chromatography, showed M_n and M_w values equalling 4000 and 5780 for II, and 4780 and 6800 for IV. Triton X-100 was purchased from Aldrich Chemical Co., and used as obtained.

Hexaethylene Glycol Monooctanoate (V). A mixture of octanoic acid (220 mg, 1.53 mmol) and $SOCl_2$ (720 mg, 6.05 mmol) was refluxed for 3 h. The excess of $SOCl_2$ was removed under reduced pressure, and the residue dissolved in 5 mL of CH_2Cl_2. To this solution was added (dropwise) a solution made from 500 mg (1.77 mmol) of hexaethylene glycol (Aldrich) dissolved in 10 mL of CH_2Cl_2/pyridine (9/1, v/v) over a period of 5 min. After additional stirring (18 h) at room temperature, the organic phase was washed three times with 10 mL of water, dried (anhydrous Na_2CO_3), and concentrated under reduced pressure. The monoester V product was isolated via silica gel chromatography using CH_2Cl_2/CH_3OH (39/1, v/v), affording 194 mg (31%) of a colorless oil: ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H), 1.28 (s, 8 H), 1.60 (m, 2 H), 2.33 (t, 2 H), 2.92 (s, 1 H), 3.66 (s, 22 H), 4.23 (t, 2 H). Anal. Calcd for $C_{20}H_{40}O_8$: C, 58.80; H, 9.87. Found: C, 58.73; H, 10.08.

Surfactant-Induced Release of Liposome-Encapsulated CF. Large unilamellar vesicles were prepared from the appropriate phosphatidylcholine or phosphatidylcholine/cholesterol mixtures (in chloroform) by standard procedures.[18] Typically, 2 mL of a chloroform solution, containing 20 mg of POPC, was placed in a test tube (13 × 100 mm) and the chloroform evaporated under a stream of nitrogen. After further drying (12 h, 23 °C, 0.05 mmHg), the resulting film was suspended in 1 mL of a 100 mM solution of 5(6)-carboxyfluorescein (CF; pH 7.4),[17] via vortex mixing. The resulting multilamellar vesicle dispersion was allowed to equilibrate for 0.5 h, subjected to five freeze–thaw cycles (liquid nitrogen), extruded (10 times) through a 0.1-μm polycarbonate filter (Nuclepore Co.), and purified via gel filtration on a Sephadex G-50 column (0.7 × 40 cm), using a 10 mM borate buffer (pH 7.4, 140 mM NaCl, 2 mM NaN_3) as the eluant. Those fractions which contained large unilamellar vesicles, prepared by this extrusion technique (LUVETs), were combined, and the final volume was adjusted to ca. 5 mL, by adding further buffer solution. Finally, this liposomal dispersion was dialyzed against 200 mL of the same borate buffer used for gel filtration (12 h, 15 °C), and allowed to reach room temperature, just prior to use.

An aliquot (10 μL) of the above vesicle dispersion was added to each of a series of test tubes (6 × 50 mm), which contained 90 μL of a given surfactant solution (the final concentration of lipid was ca. 0.5 mM as determined by phosphorus analysis[19]), and the resulting suspension agitated by vortex mixing for ca. 10 s. After the mixture was allowed to incubate for 0.5 h at 23 °C, 50-μL aliquots were withdrawn and diluted with 4 mL of borate buffer. The fluorescence was then determined by using a Turner fluorimeter (Model 112). A blank value was determined by treating 10-μL aliquots of the original vesicle dispersion with 90 μL of buffer, in the absence of detergent. A total fluorescence value was determined by complete disruption of the vesicles, by using 90 μL of a buffer solution that was 80 mM in Triton X-100. The percentage of released CF was calculated according to $I\ (\%) = 100[I_a - I_b]/I_x - I_b]$, where I_x is the 100% fluorescence intensity determined with an excess of Triton X-100; I_a and I_b are the fluorescence intensities after incubation with and without surfactant, respectively. Values of R_{50} represent the ratio of phospholipid to surfactant (or repeat unit) that is needed to release 50% of the entrapped CF from a 0.5 mM dispersion of liposomes after 30 min.

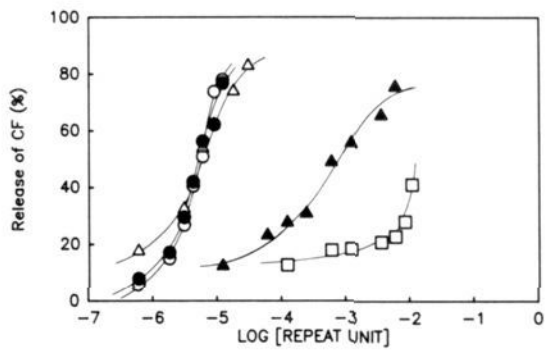


Figure 2. Percent release of CF from 0.5 mM liposomal targets made from egg PC(○), POPC(●), POPC/cholesterol (4/1) (△), POPC/cholesterol (2/1) (▲), POPC/cholesterol (1/1) (□) as a function of molar surfactant (or repeat unit) concentration of II.

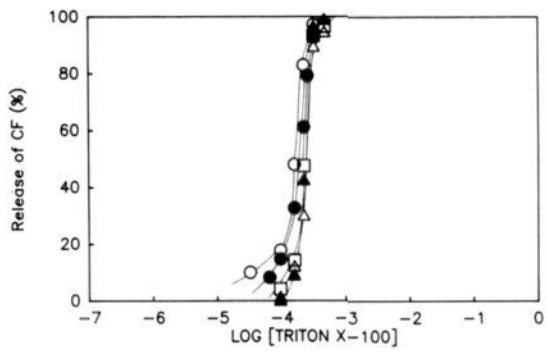


Figure 3. Percent release of CF from 0.5 mM liposomal targets made from egg PC(○), POPC(●), POPC/cholesterol (4/1) (△), POPC/cholesterol (2/1) (▲), POPC/cholesterol (1/1) (□) as a function of molar surfactant concentration of VI.

(14) The T_m value for POPC is −5 °C: DeKrujff, B.; Demel, R. A.; Slotboom, A. J.; Van Deenen, L. L. M.; Rosenthal, A. F. *Biochim. Biophys. Acta* **1973**, *307*, 1.

(15) Egg PC has a T_m that lies between −5 and −15 °C: Ladbrooke, B. D.; Chapman, D. *Chem. Phys. Lipids* **1969**, *3*, 304.

(16) Ruiz, J.; Goni, F. M.; Alonso, A. *Biochim. Biophys. Acta* **1988**, *937*, 127.

(17) Weinstein, J. N.; Ralston, E.; Lesereman, L. D.; Klausner, R. D.; Dragsten, P.; Henkart, R.; Blumenthal, R. In *Liposome Technology*; Gregoriadis, G., Ed.; CRC Press, Inc.: Boca Raton, FL, 1984; Vol. III, pp 183-204.

(18) Hope, M. J.; Bally, M. B.; Webb, G.; Cullis, P. R. *Biochim. Biophys. Acta* **1985**, *812*, 55.

(19) Regen, S. L.; Kirszenstejn, P.; Singh, A. *Macromolecules* **1983**, *16*, 335.

Table I. Selectivity Features of Membrane-Disrupting Surfactants

target membrane	surfactant R_{50} (S)[a]					
	I	II	III	IV	V	VI
egg PC	5.7 (1.5)	64 (0.58)	1.8 (0.82)	68 (0.40)	0.20 (0.65)	2.3 (1.4)
POPC	3.9 (1.0)	110 (1.0)	2.2 (1.0)	170 (1.0)	0.31 (1.0)	1.7 (1.0)
POPC/cholesterol (4/1)[b]	3.8 (0.97)	83 (0.75)	1.7 (0.77)	0.11 (0.00065)	0.18 (0.58)	1.7 (1.0)
POPC/cholesterol (2/1)[b]	0.016 (0.0041)	0.72 (0.0065)	1.7 (0.77)	<0.070 (<0.00041)[c]	0.086 (0.28)	1.9 (1.1)
POPC/cholesterol (1/1)[b]	0.0044 (0.0011)	0.032 (0.00029)	<0.069 (<0.031)[c]	<0.071 (<0.00042)[c]	0.023 (0.074)	1.7 (1.0)

[a] R_{50} represents the ratio of phospholipid to surfactant (or repeat unit) that is needed to release 50% of the entrapped CF from a 0.5 mM dispersion of liposomes after 30 min; S is the surfactant selectivity that is represented by the ratio of R_{50} for a given target to R_{50} for POPC-based liposomes. All release curves used to calculate R_{50} values were derived from a minimum of five independent experiments. Values reported for I, II, III, IV, and VI, acting on POPC membranes, represent averages calculated from 3, 5, 2, 2, and 5 release curves, respectively; standard deviations were ± 0.3, 30, 0.1, 76, and 0.2, respectively. Values reported for I, II, and VI, acting on POPC/cholesterol (1/1), are averages obtained from 3, 3, and 2 release curves, respectively, with corresponding standard deviations of ±0.0019, 0.009, and 0.1. All other R_{50} value were obtained from single release curves. [b] Molar ratio of POPC/cholesterol. [c] Limited by solubility of surfactant.

Lysis of *P. mirabilis*. A series of microcentrifuge tubes were charged with 100 μL of culture medium (heart infusion broth plus inactivated horse serum and baker's yeast extract) that contained a suspension of 4.5 × 10^7 cells of *P. mirabilis*. To each tube was then added 100 μL of an appropriate concentration of II or VI dissolved in this same medium. After each mixture was allowed to incubate for 1 h at 37 °C, aliquots were withdrawn and the concentration of remaining cells was analyzed by visual counting (light microscopy).

Results and Discussion

Membrane-Disrupting Selectivity in Model Systems. Incubation of 0.5 mM liposomal dispersions with varying concentrations of each surfactant at 23 °C generated release profiles that were similar to those previously described (Figures 2 and 3).[12,13] For purposes of comparison, we report membrane-disrupting activity as R_{50} values, where R_{50} represents the ratio of phospholipid to surfactant (or repeat unit) that is needed to release 50% of the entrapped CF from a 0.5 mM dispersion of liposomes after 30 min. In order to gauge the selectivity of each surfactant, we have chosen POPC as a standard reference. Specifically, we define a selectivity parameter, S, as the ratio of R_{50} for a given target to R_{50} for POPC-based liposomes.

Table I summarizes the results obtained for I–VI, acting on the five membranes. From these data, it is clear that *all* of the surfactants are active in disrupting lipid bilayers produced from a complex phosphatidylcholine mixture (egg PC) and from the single phospholipid POPC. The high disrupting activity that was previously found for II and IV toward POPC-based liposomes is also apparent against egg PC bilayers. While addition of 20 mol % of cholesterol to POPC bilayers had no significant effect on improving its resiliency toward I, II, and III, it did, however, considerably inhibit the disrupting action by the unsaturated polymer IV. With higher levels of cholesterol (i.e., 33 and 50 mol %), membrane stability substantially increased toward I, II, and IV, but was only modestly increased toward V; with III, significant stabilization was observed only when 50 mol % of cholesterol was used. In essence, the single-chain surfactant V showed relatively little sensitivity toward the presence of cholesterol; surfactant VI was completely insensitive. Taken together, these results clearly establish that it is, indeed, possible to effectively distinguish among lipid membranes on the basis of their cholesterol content, and that bolaphiles and supramolecular surfactants can provide significantly greater discriminating power as compared with single-chain analogues.

Previous studies have shown that the membrane-disrupting abilities of certain poly(ethylene glycol)-based nonionic surfactants do not correlate with their aggregation properties in solution, i.e., their critical micelle concentrations (CMC's).[20,21] It is noteworthy that the CMC's that characterize I–VI (1.3 × 10^{-4}, 7.1 × 10^{-6}, 3.0 × 10^{-4}, 6.8 × 10^{-6}, 1.9 × 10^{-3}, and 2.6 × 10^{-4} M, respectively) do, in fact, correlate with the R_{50} values observed for POPC. In particular, those surfactants which have lower CMC values exhibit higher membrane-disrupting activity. The *selectivity* features of these surfactants, however, show no correlation with their critical micelle concentrations.

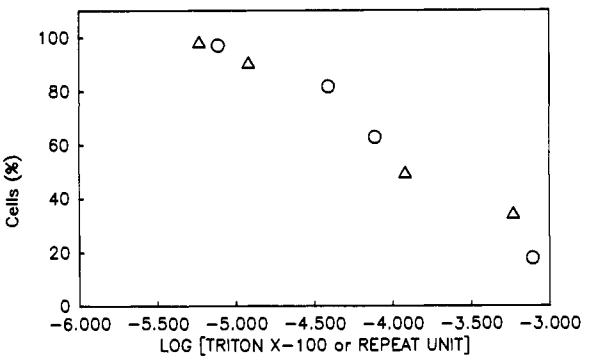


Figure 4. Lytic action by II (△) and VI (○) on *P. mirabilis* (4.5 × 10^7 cells). Cell cultures were incubated for 1 h at 37 °C in the presence of varying molar concentrations of surfactant.

Biomembrane Targets. In previous studies, we have shown that the supramolecular surfactant II is completely inactive in lysing human erythrocytes but that VI is highly active.[11] In striking contrast, II is more active than VI in disrupting POPC membranes.[12] On the basis of our present findings, we believe that the robustness of red blood cells toward II is a likely result of their high cholesterol content (ca. 50% of the total lipid) and their tight membrane packing.[22] Because bacteria are devoid of cholesterol, one might expect that such microorganisms could be lysed by II as well as by VI. Consistent with this prediction, we have found that both II and VI are capable of lysing the bacterium *P. mirabilis* (Figure 4).

Implications of These Findings. The results that are described herein have several important implications. First, the demonstration that membrane-disrupting surfactants can be created that recognize subtle differences in membrane packing, together with the fact that the outer membranes of mammalian cells, viruses, fungi, and bacteria vary in composition and packing, lends strong support to our hypothesis that molecules of this type may have therapeutic potential.[11] The ability of a supramolecular surfactant II to readily lyse a human bacterium (i.e., *P. mirabilis*) but not erythrocytes provides in vitro support for this view. Second, the alteration in selectivity of a bolaphile and a supramolecular surfactant, upon introduction of one double bond, strongly suggests that further control may be possible through appropriate chemical modification. It seems plausible, for example, that membrane-disrupting selectivity could be further modulated by adjusting the hydrocarbon chain length, increasing the amount of unsaturation, and incorporating bulky groups within the aliphatic segments. Third, the low disrupting power of I–IV toward cholesterol-rich membranes suggests that they could serve as "mild" formulating agents for the solubilization of hydrophobic drugs. Most importantly, *these results provide the first clear demonstration that*

(20) Miyajima, K.; Baba, T.; Nakagaki, M. *Colloid Polym. Sci.* **1987**, *265*, 943.

(21) Miyajima, K.; Baba, T.; Nakagaki, M. *Colloid Polym. Sci.* **1989**, *267*, 201.

(22) Portlock, S. H.; Clague, M. J.; Cherry, R. J. *Biochim. Biophys. Acta* **1990**, *1030*, 1.

modest and definable differences in membrane composition and packing can lead to large differences in lability, and that synthetic agents can be created which exploit such differences. They also reveal the fact that subtle changes in surfactant structure can result in dramatically different interactions with lipid bilayers.

Finally, it should be mentioned that the ability of a microorganism to develop resistance toward a drug that functions via membrane disruption is likely to be less than one which operates at the nuclear or cytoplasmic level. In principle, therefore, membrane-disrupting antimicrobial agents may be better suited for treating those diseases that require long-term administration (e.g., AIDS). It is noteworthy, in this regard, that recent in vitro studies have shown that the human bacterium mycoplasma *M. fermentans* can profoundly enhance the cytotoxicity of HIV-1 toward CD4$^+$ human T-lymphocytes.[23] If such synergy is found to exist in vivo, then the synthesis of membrane-disrupting anti-mycoplasma agents could represent a rational approach toward novel anti-AIDS drugs.[24]

A detailed kinetic and mechanistic investigation into the membrane-disrupting action of I–VI, as well as the design and synthesis of second-generation analogues, is now in progress. The results of these studies will be reported in due course.

Acknowledgment. We are grateful to Dr. Ferenc J. Kezdy (Upjohn Co.) for many valuable discussions and to Mr. Wally Patton (Lehigh) for technical assistance.

(23) Lo, S.-C.; Tsai, S.; Benish, J. R.; Shih, J. W.-K.; Wear, D. J.; Wong, D. M. *Science* **1991**, *251*, 1074.

(24) In preliminary studies, II has been found to completely inhibit the growth of two human strains of mycoplasma fermentans (i.e., Incognitus and PG18) at a concentration level of 63 μg/mL; complete inhibition by I required a concentration of 1000 μg/mL: Lo, S.-C.; Kotani, H.; Regen, S. L. Unpublished results. Thus, the greater anti-mycloplasma activity observed with the supramolecular surfactant correlates with its higher activity in disrupting fluid POPC bilayers.

Oxidative α Coupling of Carbonyl Compounds via the Condensation of Acylated Triazolinedione Ylides with Enolates: A Facile Synthesis of Polyacylated Olefins

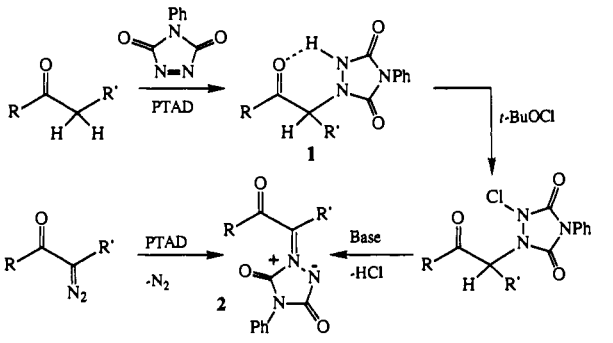
R. Marshall Wilson,* Alvan C. Hengge, Ali Ataei, and Douglas M. Ho

Contribution from the Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221-0172. *Received October 11, 1990. Revised Manuscript Received April 11, 1991*

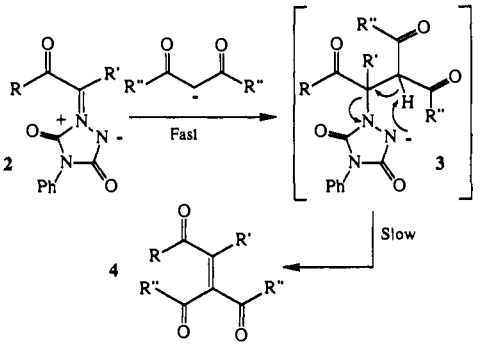
Abstract: α-Urazolyl ketones are readily oxidized to acylated *N*-phenyltriazolinedione ylides with *tert*-butyl hypochlorite. These ylides, usually as generated in situ, undergo condensations with enolate species to form acylated olefins in synthetically useful yields. The ylide derived from deoxybenzoin is sufficiently stable to be isolated and characterized, and displays the same type of condensation chemistry with enolates as do other less stable ylides that must be generated in situ. The tri- and tetraacylolefins produced by this method are susceptible to subsequent Michael addition of enolates. Intramolecular cyclization or basic cleavage of these Michael adducts leads to a variety of interesting secondary products.

N-Phenyltriazolinedione (PTAD) ylides constitute a relatively obscure class of reactive intermediates which have recently been shown to undergo extremely facile condensations with a variety of nucleophiles.[1] More recent efforts have shown that ylide precursors, α-urazolylcarbonyl compounds (**1** in Scheme I), are readily available through the reaction of a wide variety of carbonyl compounds with PTAD.[2] In an effort to extend the scope of this novel condensation chemistry of PTAD ylides, we have investigated the generation of acylated PTAD ylides (**2**) from these α-urazolylcarbonyl compounds,[3] and in this paper we report the condensation chemistry of these acylated ylides with a variety of enolate species as outlined in Scheme II and Table I.

Scheme I



Scheme II



PTAD Ylides and Their Reactions with Enolates

In a number of instances, PTAD ylides may be isolated as moderately stable, colored solids.[1,4,5] As might be expected, these more stable ylides all contain stabilizing electron-donating or phenyl substituents. In the ylides under study here, the electron-withdrawing acyl substituent tends to destabilize the ylides to the extent that they cannot be isolated unless they are stabilized by a complementary electron-donating substituent such as a phenyl group (**2**, R′ = Ph).[4] Thus, in most of the condensation reactions studied here (Table I), the ylides were not isolated, but were prepared in situ. The α-urazolylcarbonyl compounds were oxidized

(1) (a) Wilson, R. M.; Hengge, A. C. *Tetrahedron Lett.* **1985**, *26*, 3673. (b) Wilson, R. M.; Hengge, A. C. *J. Org. Chem.* **1987**, *52*, 2699.
(2) Wilson, R. M.; Hengge, A. C.; Ataei, A.; Chantarasiri, N. *J. Org. Chem.* **1990**, *55*, 193.
(3) Wilson, R. M.; Hengge, A. C. *J. Org. Chem.* **1990**, *55*, 197.
(4) Theis, W.; Bethäuser, W.; Regitz, M. *Tetrahedron* **1985**, *41*, 1965. Theis, W.; Bethäuser, W.; Regitz, M. *Chem. Ber.* **1985**, *118*, 28.
(5) Wilson, R. M.; Chantarasiri, N. *J. Am. Chem. Soc.* **1991**, *113*, 2301.

0002-7863/91/1513-7240$02.50/0