Hydrolysis of Nonionic Ester Surfactants Facilitated by Potassium β-Glycyrrhizinate: Implication of Catalytic Functions Played by the Carboxyl Groups

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ABSTRACT: Two different types of methyl esters of β-glycyrrhizinate (GK2) have been prepared to delineate the mechanism of the catalytic action of each carboxyl group of GK2 in the hydrolysis of nonionic fatty acid surfactants. In the acidic pH region of less than 4, the hydrolysis is catalyzed by the carboxyl groups of the sugar moiety, while in the close-to-neutral acidic region, the carboxyl group at the hydrophobic triterpenoid end becomes important in catalysis.
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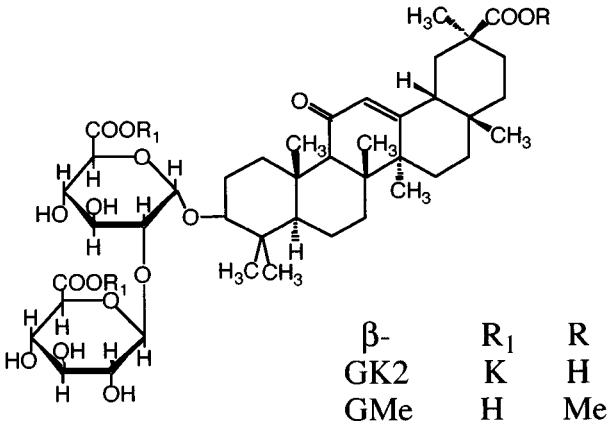
KEY WORDS: Acid catalysis, methyl glycyrrhizinates, nonionic surfactant ester hydrolysis.

Dipotassium β-glycyrrhizinate (GK2) serves as an anionic surfactant as well as an antiallergic and antiinflammatory drug in eye lotions and injectable formulations (1). The structure of GK2 consists of two parts, a hydrophobic triterpenoidal part and a hydrophilic di-D-glucuronic (sugar) part. The three carboxyl groups in GK2 can be classified into two groups: the one present in the hydrophobic triterpenoid has a high pKa value of 8.5 (2), and the other two, one on each hydrophilic glucuronic part, are more acidic and not strictly distinguishable from each other in catalytic activity because of similarity of their acidities (pKa = 3.3 and 4.7). Scheme 1 depicts the structure of GK2 and its related derivatives, and their abbreviation symbols also are shown.



SCHEME 1

Recently, we found that GK2 catalyzes the hydrolytic bond cleavage of nonionic fatty acid ester surfactants, such as polyoxyethylene(60) hydrogenated castor oil (HCO-60, Scheme 2), even in the pH range of 3 to 6 (3). Because HCO-60 is nontoxic, it has been occasionally employed in pharmaceutical formulations for solubilizing oil-soluble ingredients (4,5). The ester itself is virtually nonsusceptible to hydrolysis without GK2 in citrate buffer, indicating that a short-chain carboxylic acid has no influence on the rate (6). HCO-60 itself was reported to form a single micelle, spherical or nearly spherical, with a critical micelle concentration (CMC) of 0.0263 mM and an aggregation number of 92 (7). GK2 constitutes a rod-like micelle with a CMC of 0.68 mM and an aggregation number of 130 (pH 5.0) (8). Their mixture, used in these experiments, is expected to form a mixed anionic/nonionic micelle, which is responsible for the observed catalytic activity (2). Thus, we proposed that a GK2 molecule and a fatty acid ester molecule produce an energetically favorable complex for catalysis *via* hydrophobic interactions in the micellar aggregates that brings the ester carbonyl group in close contact with one of the three carboxyl groups in the GK2 molecule.

In this paper, we report the results of a mechanistic study of the catalytic action of the individual carboxyl groups in the hydrolysis of fatty acid ester surfactants by GK2. To elaborate the functions of the GK2 molecule, we synthesized two different kinds of methylated GK2.

EXPERIMENTAL PROCEDURES

Materials. GK2, glycyrrhetinic acid, and stearyl glycyrrhetinate from Maruzen Seiyaku (Hiroshima, Japan) were used as

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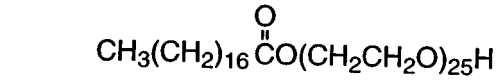
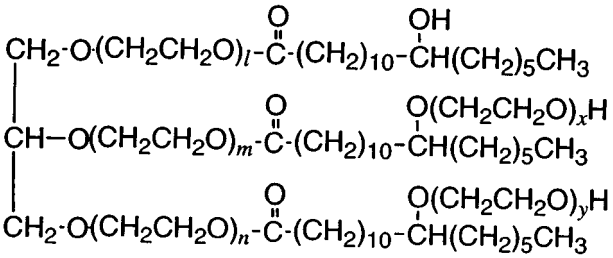
POES



HCO-60



$l + m + n + x + y = 60$

SCHEME 2

received. Stearic acid was purchased from Tokyo Kasei (Tokyo, Japan). Reagent-grade HCO-60 and polyoxyethylene(25) glycol monostearate (POES, Scheme 2) were obtained from Nikko Chemicals (Tokyo, Japan). Commercially available buffers, including citric acid, were extra pure and used as received.

Monomethylated GMe was prepared from the starting material GK2 as follows: first, the diglucuronic carboxyl groups were protected with benzylation (Bn) by allowing 5 g (5.56 mmol) of GK2 to react with more than two equivalents of benzyl bromide (3.3 mL) in 100 mL dimethylformamide (DMF) at 60°C for 3 d. Solvent removal and then chromatographic separation on a silica gel (Wakogel 60, purchased from Wako Pure Chemicals; Osaka, Japan) with $CHCl_3$–MeOH (10:1, vol/vol) as an eluent gave 3.35 g (60% yield) of the dibenzylated GK2 at the sugar part (GBn2) and a small amount (*ca.* 5%) of the tribenzylated GK2 (GBn3); GBn2: m.p. 164–166°C; δ (DMSO-d_6): 7.42 (*s*, 4 H, aromatic H), 7.38 (*s*, 6 H aromatic H), 5.19 (*s*, 4 H, benzylic CH_2) , 1.36-0.66 (7 *s*, 21 H, Me). GBn3 recrystallized from $CHCl_3$–hexane: m.p. 152–155°C; δ (dimethylsulfoxide, DMSO-d_6): 7.39-7.31 (6 *s*, 15 H, aromatic H) , 5.19 (*s*, 6 H, benzylic CH_2), 1.33-0.62 (7 *s*, 21 H, Me); splittings of signals due to the phenyl protons in GBn2 and GBn3, a 2:3 doublet and a multiplet, respectively, indicate that the dibenzylation takes place selectively with respect to the sugar carboxyl groups. Subsequently, the triterpenoidal carboxyl group in GBn2 (3.35 g, 3.34 mmol) was methylated at 0°C in 50 mL of dry CH_2Cl_2 with 1.1 equivalent of trimethyloxonium tetrafluoroborate and 1.1 equivalent of *N*-ethyl-diisopropylamine as a base. After solvent evaporation, chromatographic separation on a silica gel column with $CHCl_3$–MeOH (10:1) afforded 1.94 g (57.2%) of the desired intermediate (GBn2Me): m.p. 146–148°C; anal. calcd. for $C_{57}H_{76}O_{16}$: C, 67.30; H, 7.53; found: C, 67.05; H, 7.71; δ(DMSO-d_6): 7.38 (*s*, 4 H, aromatic H) , 7.34 (*s*, 6 H, aromatic H), 5.18 (*s*, 4 H, benzylic CH_2), 3.64 (*s*, 3 H, OMe), 2.15-0.62 (7 *s*, 21 H, Me). Finally, the protective benzyl moieties in GBn2Me (1.03 g, 1.01 mmol) were removed by 10% Pd/C-catalyzed hydrogenolysis in 20 mL MeOH. Catalyst filtration and solvent evaporation gave the sole product GMe as white powder (570 mg) in 67% yield; dec. 190–195°C; δ (DMSO-d_6): 3.62 (*s*, 3H, OMe), 1.33-0.67 (7 *s*, 21 H, Me) . The product thus obtained showed a single spot on a thin-layer chromatogram (TLC) and was satisfactorily pure for our purpose.

The dimethyl ester of the sugar part (GMe2) was prepared by reaction of GK2 (5 g, 5.56 mmol) with dimethyl sulfate (4 g, 28 mmol) in 100 mL DMF for 24 h at room temperature. After solvent evaporation under vacuum, the resultant residue was subjected to column chromatography on a silica gel column with $CHCl_3$–MeOH (5:1), affording 3.3 g (70%) of GMe2; dec. 220–225°C; δ(DMSO-d_6): 3.7 (*d*, 6 H, OMe), 1.34-0.67 (7 *s*, 21 H, Me). The above two compounds, as expected from their high hydrophobicity, were not surface-active. All terpenoidal proton peaks are still not completely assigned because of their structural complexity. A few peaks that are useful for confirming purity were picked out and summarized above. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNMA400 FT NMR spectrometer (Tokyo, Japan).

Kinetics of hydrolysis of POES and HCO-60. Hydrolyses were performed at 70°C in 0.1 M buffer solutions that contained 1.1 mM GK2 derivatives and 0.56 mM HCO-60 or 1.43 mM POES surfactant esters, unless otherwise stated. Product analyses were performed by employing fluorescent high-performance liquid chromatography (HPLC) analysis of 9-anthrylmethyl stearate and 12-hydroxystearate, which were derived from the fatty acids produced and the 9-anthryldiazomethane reagent. Further details on the analytical method are given elsewhere (3). The extent of the hydrolysis thus obtained has been summarized in the figures shown later. The catalytic activities of glycyrrhetinic acid, its stearyl and malonyl esters, stearic acid, and salicylic and acetic acids were also examined, and the results are summarized in Table 1. Fluorescence spectra were recorded on a Shimadzu RF-535 fluorescence HPLC monitor at an excitation wavelength of 365 nm and emission at 416 nm.

CMC measurements. The CMC values were determined in an ordinary way (9), i.e., by a dye method with Rhodamine 6G or by a surface-tension method with a Wilhelmy-type surface tensometer ST-1, Shimadzu (Kyoto, Japan), as follows. POES: 1.0 mM; 1/1 POES/GK2: 0.30 mM; 1/1 POES/GMe: 0.16 mM at pH 7.0 in 0.1 M phosphate buffer.

Computer-aided molecular mechanics calculations. Computational mechanics calculations were performed with the aid of a CAMD system from a software package of CHARMm Ver22/QUANTA Ver 3.3 (Molecular Simulation

TABLE 1
Catalytic Activities of Dipotassium β-Glycyrrhizinate (GK2) and Long- or Short-Chain Carboxylic Acids for Hydrolysis of HCO-60 to 12-Hydroxystearic Acid[a]

Catalyst	Presence of carboxyl group with: High pKa	Low pKa	Catalytic activity (rel. rate)
GK2	Yes	Yes	Yes (4.2)
Glycyrrhetinic acid[b]	Yes	No	Yes (5.0)
Stearyl glycyrretinate	No	No	No (1.0)
Lauroyl glutamate	Yes	Yes	Yes (4.8)
Stearic acid	No	Yes	Yes (6.0)
Salicylic acid	No	Yes	No (1.0)
Acetic acid	No	Yes	No (1.0)

[a]Conditions: [catalyst] = 1.1 mM; polyoxyethylene(60) hydrogenated castor oil (HCO-60) = 0.56 mM; 70°C; pH 4.9.
[b]No diglucuronic acid moieties.

Inc., San Diego, CA) on hardware of INDIGO R-4000 (Silicon Graphics Corporation, Mountain View, CA) until total energies at the global minima were attained.

RESULTS AND DISCUSSION

Table 1 shows the catalytic activity of a variety of GK2 analogs for the hydrolysis of HCO-60 (pH 4.9); GK2 and glycyrrhetinic acid, which both possess one carboxyl group in the triterpenoid part, exerted a rate-enhancing effect, but virtually no rate-enhancement was observed with stearyl glycyrrhetinate, providing strong evidence that the presence of the triterpenoid carboxyl group affects the hydrolysis. Table 1 also indicates the effectiveness of long-chain carboxylic acids. Even if all data are taken into consideration, no definite conclusions could be drawn about the function of the carboxyl groups in the sugar part.

To unequivocally address their catalytic behaviors in hydrolysis, we have methylated GK2 at the carboxyl groups on the diglucuronic unit or at the carboxyl group on the triterpenoid unit. Their effects on the hydrolyses of POES and HCO-60 in aqueous buffered solutions at various pH values were compared with that of GK2 itself. Typical plots for POES at pH 5.0 are depicted in Figure 1A. Generally, the percentage conversion increased linearly with reaction time within the range of low conversion of less than 10%. The rate increases linearly with increasing GK2 concentration in the 0.5–1.4 mM range. At high conversions, the plots deviate upward, which we attribute to autocatalysis by the long-chain fatty acid products, such as stearic acid. Short-chain carboxylic acids, such as acetic and citric acids, exhibited no definite activity for hydrolysis.

Figures 2 and 3 depict the plots of percentage conversion for POES and HCO-60, respectively, at a given time as a function of pH. Below pH 3, the rates increased exponentially with increasing acidity of the aqueous buffered solutions, implying that surfactant ester hydrolysis is hydroxonium ion-catalyzed in this pH region, as has been established for simple esters (10). At every pH, the reactions are slowest in the

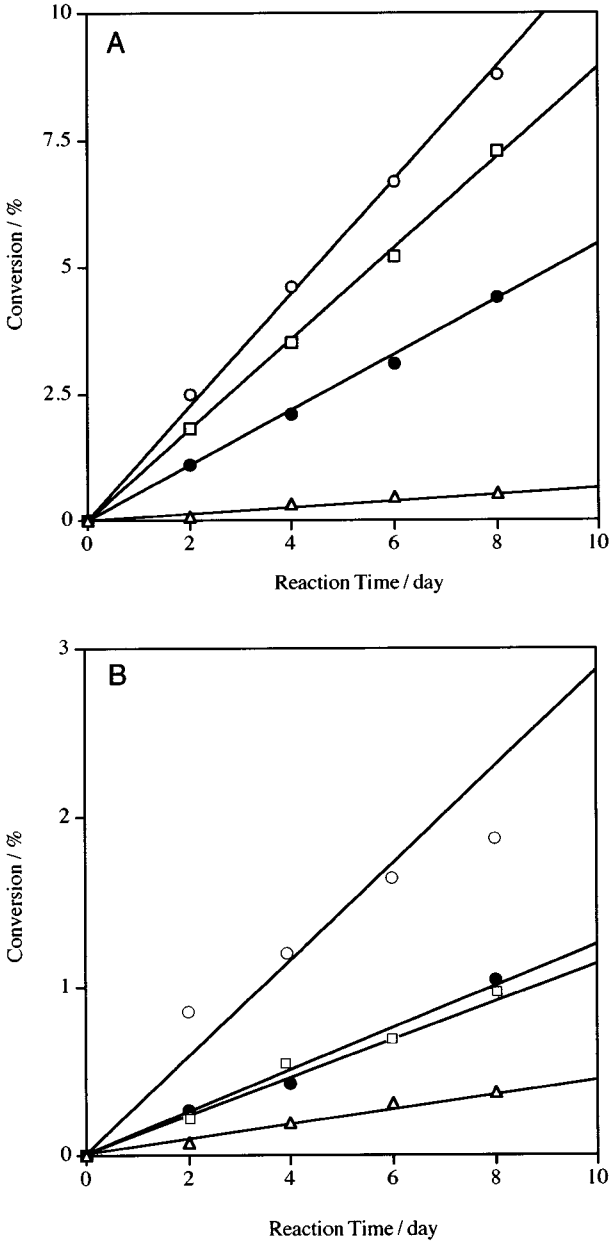


FIG. 1. Plots of percentage conversion vs. reaction time for glycol monostearate (POES) hydrolysis in the absence (△) and presence of GMe2(○), GMe(□), and GK2(●). [POES] = 1.43 mM, [catalyst] = 1.1 mM; 70°C; (A) pH 5.0 (citrate buffer); (B) pH 6.0 (citrate buffer).

absence of any catalyst. However, the hydrolysis rates for different GK2 derivatives depend upon the position of methylation.

In the more acidic region, less than pH 4.5, where GK2 probably exists in the monoanionic or neutral form, the rates decrease in the order: GMe > GK2 > GMe2. The GMe catalyst, with two sugar carboxyl groups of high acidity left unmethylated, is more active than GK2 itself, while the GMe2 catalyst, bearing a triterpenoid carboxyl group of low acidity left unmethylated, is less active than GK2. At low pH, the

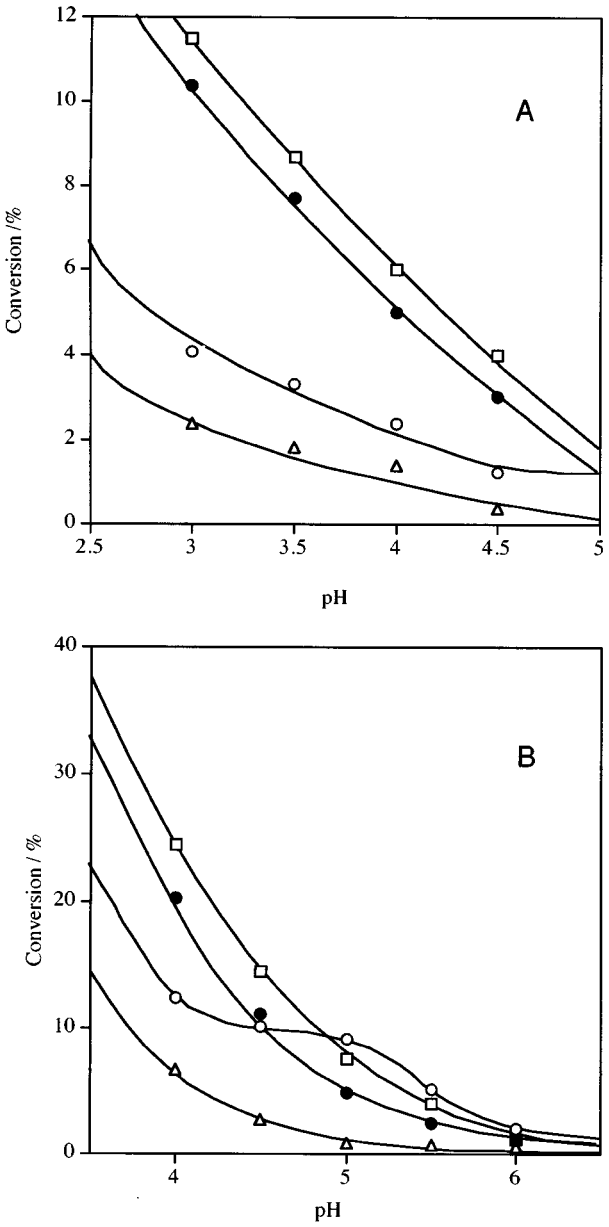


FIG. 2. The pH effects on POES hydrolyses in the absence (△) and presence of GMe2 (○), GMe (□), and GK2 (●). [POES] = 1.43 mM, [catalyst] = 1.1 mM, 70°C; (A) formate buffer (pH 3.0–4.0) and acetate buffer (pH 4.5), 2 d; (B) citrate buffer, 8 d.

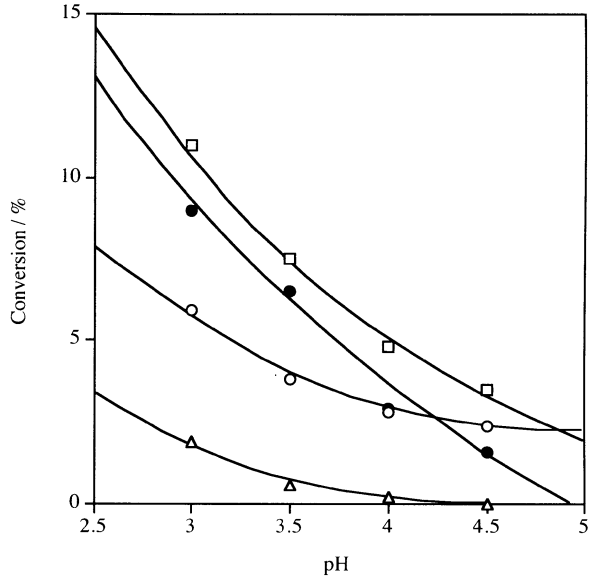
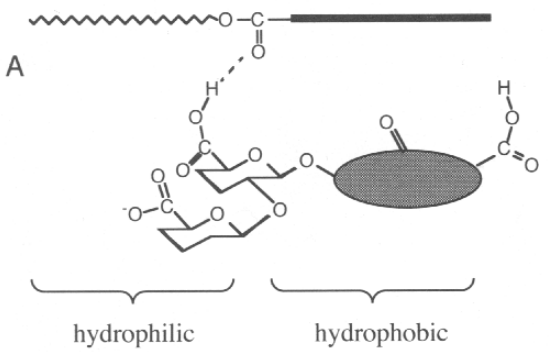


FIG. 3. The pH effects on HCO-60 hydrolyses in the absence (△) and presence of GMe2 (○), GMe (□), and GK2 (●). [HCO-60] = 1.43 mM, [catalyst] = 1.1 mM, 70°C, 0.1 M formate buffer (pH 3.0–4.0) and acetate buffer (pH 4.5) , 8 days.

undissociated carboxyl groups in the sugar part are more essential for catalysis. Methylation at the sugar carboxyl groups interferes with their capacity to either hydrogen-bond or protonate the ester carbonyl oxygen. The observation that GMe is always more effective than GK2 throughout the pH range below 5.5 suggests that the hydrophobicity of the catalyst itself is a primarily significant factor for ester activation because the major hydrolysis process would occur in a hydrophobic environment, which would strengthen hydrogen bonding interactions that make significant contributions to transition state stabilization (11); the mixed micellization of POES with GMe is capable of providing a more hydrophobic micellar environment than that with GK2, judging from their surface tension and CMC values. Figure 4A shows the most plausible structure of the association complex formed in acidic solution, where the sugar carboxyl participates in hydrogen-bonding to the ester carbonyl oxygen (3).

The rate follows a different sequence at higher pH: GMe2 > GMe > GK2 at pH 5 and 5.5, and GMe2 > GK2 ≒ GMe at pH 6.0 (Fig. 1B), revealing that the catalytic efficiency of GMe relative to GK2 decreases with increasing pH. In this closer-to-neutral acidic pH region, where GK2 exists predominantly in its dianionic form, the presence of the yet undissociated triterpenoid carboxyl group is responsible for the accelerated reaction through hydrogen bonding to the ester carbonyl oxygen in the micellar core (Fig. 2B). Thus, participation of the terpenoidal carboxyl group becomes important for catalysis in neutral media, particularly, in a hydrophobic surrounding. The structure depicted in Figure 4B corresponds to a complex in which the triterpenoidal carboxylic acid hydrogen-bonds to the carbonyl oxygen.

To gain theoretical insight into the structural feature of GK2/POES aggregates, molecular mechanics calculations for a number of force fields were made, assuming an aggregate composed of two GK2 molecules in the free form and one POES molecule, with various possible orientations and dispositions. Calculations were iteratively made until the global minimum was attained. They showed that, in the energetically optimized structure shown in Figure 5, the hydrophilic sugar part should be directed to the hydrophilic domain of POES or its aggregate, while the hydrophobic triterpenoid should be



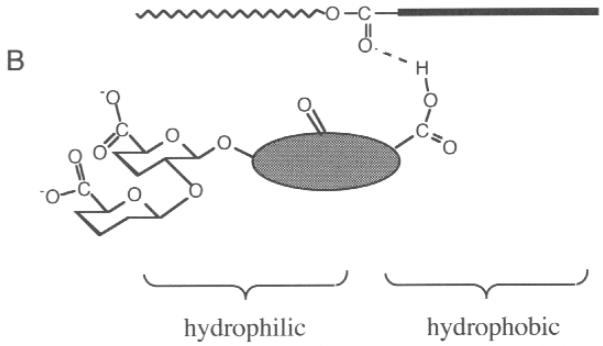


FIG. 4. Conceptual representation of the complexation between GK2 and POES. (A) Corresponds to the reaction at a low pH, (B) at a high pH.

directed to the hydrophobic domain of POES. The terpenoid carboxyl of the GK2 molecule on the underside of the POES molecule hydrogen-bonds to the carbonyl oxygen of POES, although this model, placed in vacuum, overestimates hydrogen-bonding interactions btween the sugar part and the polyoxyethylene chain. Calculations for the 1:1 GK2/POES complex exhibit a similar structural feature to that of the 2:1 complex except for the absence of the upper GK2 molecule; the hydrogen-bonding between the terpenoid carboxyl and the ester carbonyl oxygen is maintained.

Finally, we considered the catalytic ability of the GK2 derivatives at pH 7. Figure 6 shows that the kinetic behaviors of GK2 and GMe2 are comparable in the absence of catalyst, suggesting that the hydrolysis mechanism changed from GK2 acid catalysis to base catalysis. Phosphate buffer base employed, rather than hydroxide anion, appears to play a major role in catalysis because, when no buffer was used, little or no rate acceleration was observed, as anticipated from extrapolating the line in Figure 2B to pH 7.

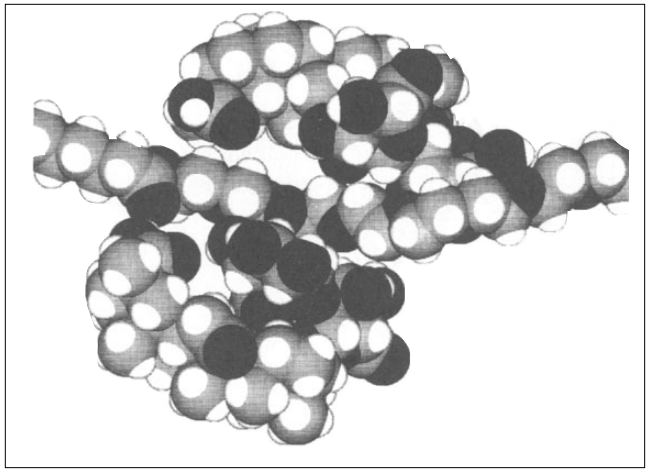


FIG. 5. Computer-generated space-filling representation of complexation between a POES and two GK2 molecules by using molecular mechanics calculations.

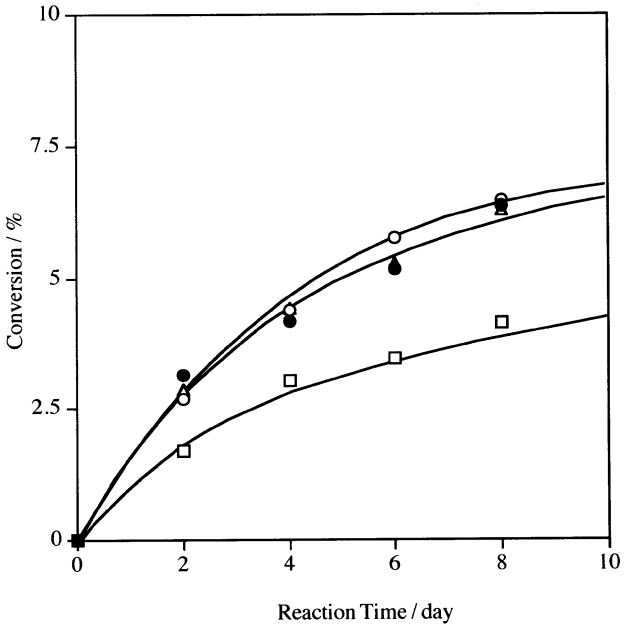


FIG. 6. The pH effects on POES hydrolyses in the absence (△) and presence of GMe2 (○), GMe (□), and GK2 (●). [POES] = 1.43 mM, [catalyst] = 1.1mM; 70°C; pH 7 phosphate buffer.

bility to base-catalyzed demethylation, giving rise to GK2 at

Meanwhile, GMe (CMC: 0.6 mM at pH 7) (8) causes a small rate retardation, probably because it has a significant tendency to construct a hydrophobic micellar core and thereby can accommodate surfactant esters into it. In the core, unfortunately, no carboxylic acid group exists to serve a catalytic role, thus resulting in a diminution of the hydrolysis rate. On the other hand, the inner core of the mixed micelles with GK2 (CMC: 1.1 mM at pH 7) (8) seems to be of a rather hydrophilic loose structure to allow buffer base catalysis.

The entire lack of catalytic activity of GMe2, which is still more hydrophobic than GMe, is probably due to its suscepti-

the early stage of the reaction. In fact, TLC analysis indicated the complete disappearance of GMe2 within 24 h, but GMe remained unhydrolyzed.

In conclusion, the use of GK2 derivatives, methylated at the different carboxyl positions, enabled us to better understand the mechanism of the GK2-catalyzed hydrolysis of nonionic ester surfactants.

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