method was applied. MTT assay is dependent on the cellular reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. 2×10^3 3LL or B-16 cells, 2×10^4 MEF(mouse embryo fibroblast) cells were inoculated in each well of flatbottomed 96-well microtiter plates in 0.18 m*l* of culture medium to which 0.02 m*l* of 10× concentrated drug or medium was added. On the 4th day, the media from the plates was aspirated completely and 50 μl of the MTT solution (1 mg/m*l*) was added to each well and incubated at 37 °C for a further 4 h. Following the incubation, to majority of the MTT solution was aspirated, in order not to disturb the formazan crystals, and 50 μl DMSO was added to each well and plates were placed on a plate shaker for 5 min and absorbance was read at 570 nm with a enzyme-linked immunosorbent assay reader.[20]

Biological activities of polymers synthesized in this study expressed by ID_{50} are summerized in Table 2. The ID_{50} values of DIVEMA, AADHP and AMDHP for normal mouse embryo fibroblasts were 765, 1587, and 1768 μg/m*l* respectively. There were no striking differences between ID_{50} values for normal and neoplastic cells; the ID_{50} values in most cases, *in vitro* ranged from 1300 to 2500 μg/m*l*. The anticancer effects of DIVEMA *in vivo* have been speculated to be mediated via a macrophage system[21-24] which cannot be reflected by simple direct cytotoxicity *in vitro*, as shown by this experiment. Thus these results also support the findings reported by others that the cytotoxic activity of DIVEMA can not be differentiated between normal and neoplastic cells *in vitro*. Studies on the anticancer effect of DIVEMA and the copolymers synthesized in this study *in vivo* are currently in progress.

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Catalytic Effects of Anion-Exchange Resins on the Ethylation of Ethyl 2-Ethylacetoacetate

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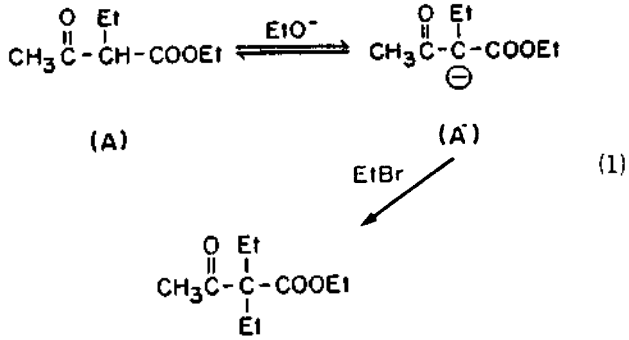
Synthetic polymers have been extensively employed as catalysts for organic reactions. The catalysis by the polymers may be attributed to the increased effective concentrations of reactants bound on the polymer,[1] effective pH on the polymer domain which is different from that in the bulk medium,[2] or the hydrophobicity created on the surface of the polymer.[2] In addition, ion-exchange resins catalyze some organic reactions by acting as heterogeneous sources of acids and bases.[3]

Table 1. Ethylation of Carbanion A⁻ with EtBr in the Presence of Various Anion-Exchange Resins[a]

Dowex resin[b]		yield (%) for ethylation of A⁻[d]
type	active group	
1X1-100	I	20
1X2-100	I	46
1X4-50	I	24
1X8-50	I	22
2X8-50	II	4
WGR-2	III	4
SBR-OH	IV	95[e]
no resin added		3

[a]Reactions were carried out at 25 °C with 0.1 g A⁻, 3 equivalent EtBr, and 2 g resin in 6 ml 1:5 (v/v) EtOH-toluene. [b]The resins differ in the degree of cross-linkage and dry mess. Detailed information may be obtained from the Sigma catalog. [c]I: Ph-CH_2-$N(CH_3)_3^+Cl^-$, II: Ph-CH_2-$N(CH_3)_2(CH_2CH_2OH)^+Cl^-$, III: Ph-$CH_2$-$N(CH_3)_2H^+Cl^-$, IV: Ph-$CH_2$-$N(CH_3)_3^+OH^-$. [d]The O-ethylated product was not formed. [e]The yield was 50% and 70% when the amount of added resin was 0.5 g and 1.0 g, respectively.

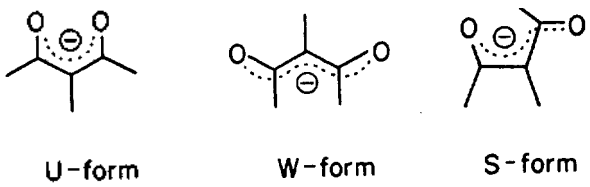
We have made various attempts to develop catalytic systems for the alkylation of sterically hindered carbanions.[4] In this paper, we report the catalytic action of commercially available anion-exchange resins on the ethylation (Eq. 1) of the carbanion derived from ethyl 2-ethylacetoacetate (A) with ethyl bromide to produce ethyl 2,2-diethylacetoacetate.



(1)

As summarized in Table 1, the yield for the ethylation of A⁻ was considerably improved by the addition of anion-exchange resins.[5]

Like other nonrigid β-dicarbonyl anions, A⁻ would exist in three major conformations: U-, W- and S- shapes.[6] Examination of a space-filling model indicates that the anionic carbon of A⁻ is most exposed in the W- conformation and least exposed in the U- conformation to the attack of external reagents. Association of the carbanion with a metal ion would lower the nucleophilicity of the anionic center. In addition, the metal association prefers the U-form, decreasing the amount of the more productive W- or S- form.



Previously, ethylation of the carbanions derived from ethyl acetoacetate or A were studied in various solvents.[6] Thus, the yield for the ethylation of A⁻ (5% in tetrahydrofuran (THF)) was much smaller than that of the carbanion (83% in THF) of ethyl acetoacetate. This was ascribed to the much greater steric hindrance imposed on the carbanionic center in A⁻.[4] It was also found that the yield for the ethylation of A⁻ was noticeably improved when dimethyl sulfoxide (DMSO) was used as the solvent (80% yield) or when a crown ether (15-crown-5) was added to the reaction mixture (100% yield in DMSO and 71% yield in THF).[4] By selectively interacting with cations, DMSO and the crown ether would increase the effective radii of the cations and, thus, lower their charge densities.[4] Consequently, the electrostatic effects exerted by the counter-cation on carbanion A⁻ would be weakened, leading to the enhanced nucleophilicity of the carbanion. Moreover, dissociation of the counter-cation from A⁻ would increase the concentration of more reactive W- or S-conformation, relieving the severe steric hindrance imposed on the reaction site.

The catalytic effects (Table 1) of anion-exchange resins can be also explained in terms of the weakened electrostatic interaction of anion A⁻ with the counter-cation. Anion A⁻ dissolved in the solution would be attracted onto the cationic surfaces of the anion-exchange resins by electrostatic interaction. Since the cationic sites on the resins are alkylated ammonium ions while the counter-cation of A⁻ in the bulk medium is sodium ion, the charge density of the counter-cation would be reduced when anion A⁻ is adsorbed on the resins. Consequently, A⁻ would become partly naked and acquire conformational freedom on the surface of the resins as in DMSO or in the presence of a crown ether.

The yield is considerably greater when the anion of the anion-exchange resin is hydroxide ion instead of chloride ion (Table 1). This may be taken to indicate that the adsorption of anion A⁻ on the polymer surface is inhibited by chloride ion, suggesting the greater affinity of chloride ion to the cationic polymer compared with hydroxide ion.

Anion-exchange resin types 2X8-50 and WGR-2 do not exert catalytic effects in constrast to the other resins. The hydroxyl group present in the quaternary ammonium ion of resin 2X8-50 can interact with A⁻ through hydrogen bonding. This could abolish the catalytic effects of the quaternary ammonium ions. The cationic site in resin WGR-2 is derived from a tertiary amine and contains a proton. This proton would be abstracted by the carbanionic center of a bound A⁻, thus quenching the nucleophilicity of A⁻.

The catalytic effects observed in the present study are attributable to the production of naked carbanions on the surface of anion-exchange resins containing quaternary ammonium ions. The catalytic feature disclosed by the present investigation may be applied to the synthesis of many other substances.

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5. Dowex anion-exchange resins were purchased from Sigma Chemical Co. The sodium salt of A^- was obtained by reacting A with sodium ethoxide, and the yield and product distribution for ethylation of A^- with EtBr conducted under various conditions were measured by ^1H nmr and gas chromatography as reported previously.[4] The progress of ethylation was checked daily for up to 5-7 days, and it was found that the maximum yield was attained within 3 days. The yield and product distributions were not affected significantly by the speed of stirring of the reaction mixture.
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Computer Graphics / Molecular Mechanics Studies on Non-Classical β-Lactam Structures*

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In the preceding paper[1] we described calculation of geometries of several representative β-lactam antibiotics by computer graphics and molecular mechanics (CG/MM) energy minimization, and found reasonable agreements between the calculated geometries and the X-ray crystal structures. The discrepancies in the compared geometries between the calculated and the X-ray structures were attributed to the crystal packing effects. It was concluded that the CG/MM energy minimization procedures based on the MM-2 and AMBER force fields could generate reasonable β-lactam geometries, especially in terms of the molecular parameters considered to be critical for the biological activities, *e.g.*, the pyramidal character of the amide nitrogen and the Cohen distance.[2]

In the recent years there have been a number of attempts to design non-classical β-lactams or their structural analogs.[3-14] Therefore, it seemed very interesting to examine the possibility of predicting the critical molecular parameters of some of these and other hypothetical compounds by using the CG/MM method. Thus, we have selected a number of novel β-lactam analogs, *i.e.* compounds **1-8**, and produced calculated structures by the procedures previously described.[1] The characteristic molecular parameters of the calculated geometries of compounds **1** through **8** are listed in Table 1.

Table 1. Molecular Parameters for "Non-classical β-Lactams"

Compound	Bond angle around N (Deg) MM-2	Bond angle around N (Deg) AMBER	Cohen Distance (A)* MM-2	Cohen Distance (A)* AMBER
1	335.6	306.0	3.485	3.384
2	341.9	327.5	3.112	3.080
3	341.1	325.0	3.967	4.001
4	355.7	344.2	3.098	2.887
5	357.6	352.8	3.441	3.439
6	357.6	354.0	3.115	3.030
7	360.0	356.7	2.995	2.789
8	360.0	356.5	2.980	2.784

*Distance between the oxygen atom of the β-lactam amide functionality and the carbon atom of the carboxy group.

The γ-lactam **1** was reported in the patent literature to have antibacterial activities.[3] The calculated molecular structure of **1** shows a substantial degree of pyramidal character (335.6 and 306.0 °C) and a reasonable Cohen distance (3.485 and 3.384 A), thus meeting the minimum structural requirements for biological activity. Baldwin, *et al.* synthesized the phenoxyacetyl derivative of the γ-lactam **3**, and found it to have no antibiotic activity. The X-ray crystallographic studies of the corresponding *t*-butyl ester showed the sum of the angles around nitrogen was 326° and the Cohen distance 4.1 A.[4,5] The calculated structure of compound **3** also indicated a pyramidal lactam nitrogen (341.1 and 325.0°) and the Cohen distance being a bit too long (3.967 and 4.001 A) to show the bioactivity. Based on the calculated molecular parameters, however, the epimeric structure **2** may be predicted to have a more desirable Cohen distance (3.112 and 3.080 A), and therefore to possess some biological activities.

Synthesis of the phenoxyacetyl derivative of compound **4** was reported to show "weak but real" antibacterial activity against both gram positive and gram negative bacteria.[6] The calculated geometry indicates a low degree of pyramidality (355.7 and 344.2°) and a low limit number (3.098 and 2.887 A) of the desirable Cohen distance range (3.0-3.9 A). The phenoxyacetyl derivative of structure **5** was reported to have a planar amide nitrogen and no antibacterial activity.[7,8] The calculations show a reasonable Cohen distance (3.441 and 3.439 A), but a virtually planar geometry around the amide nitrogen (357.6 and 352.8°) in accord with the experimental

* Dedicated to Professor A. Ian Scott on the occasion of his 60th birthday.