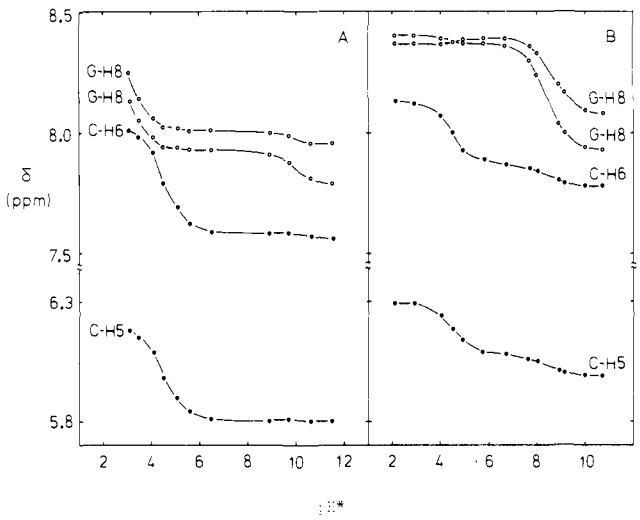

Figure 2. Chemical shift (δ) vs. pH* of the nonexchangeable base protons of the guanine (-O-) and cytosine (-●-) residues of d(G-C-G) (A) and d(G-C-G)·*cis*-Pt (B). Spectra were obtained from 2 mM solutions in D_2O at 25 ± 1 °C; tetramethylammonium nitrate was used as an internal reference (δ 3.18).

termined by a detailed analysis of the pH dependence of the chemical shifts of the base protons of d(G-C-G)·*cis*-Pt. For comparison, the pH dependence of the chemical shifts of the base protons of free d(G-C-G) was also determined (Figure 2). It is seen that all resonances of the base protons shift to lower magnetic field upon binding of *cis*-Pt. The sigmoid curves obtained upon plotting the chemical shifts of the base protons against pH* (Figure 2) can be ascribed to protonations or deprotonations of the heterocyclic nitrogens of the purine and pyrimidine bases.[4,5] The change in chemical shift for protons of free d(G-C-G) at pH 10.0 is ascribed to deprotonation of the N1 of both guanines and the chemical shift change at pH* 4.5 to protonation of the cytosine N3.[10,11] At low pH* the guanine H8 resonances start to shift strongly to lower field. This is attributed to protonation of the guanine N7 atoms, which is known to occur at about pH* 2.3.[11] So that depurination of d(G-C-G) could be avoided no spectra of this compound were recorded below pH* 3.

In d(G-C-G)·*cis*-Pt there are only two chemical shift changes with pH*: one at pH* 4.5 due to protonation of the cytosine N3 and one at pH* 8.5, which is ascribed to deprotonation of the guanine N1 atoms. Upon lowering the pH* from 3 to 2, no further chemical shift change is observed for any proton of d(G-C-G)·*cis*-Pt, indicating that protonation of the guanine N7 atoms does not occur. Undoubtedly, this is due to the fact that both guanine N7 atoms are coordinated to platinum. From the chemical shift changes at pH* 4.5 it is clear that the cytosine N3 atom is not bound to *cis*-Pt. The apparent pK_a for the deprotonation of the guanine N1 (8.5) is significantly lower than in the free trinucleotide (10.0). Such a lowering of the pK_a is common in guanine compounds with *cis*-Pt bound at their N7 atoms.[4,5,10]

Although the cytosine base is not involved in binding to *cis*-Pt, the resonances of the cytosine H5 and H6 protons are considerably shifted downfield. Chelation of *cis*-Pt by the two guanines of d(G-C-G) brings the guanines close together, so the cytosine can no longer be part of a stacked arrangement. As a result, shielding of the cytosine H5 and H6 protons by the aromatic rings of the guanine bases, which normally occurs in the stacked arrangement of the bases in oligonucleotides, will decrease, resulting in downfield shifts for these protons. The conformation of d(G-C-G)·cis-Pt could in some sense resemble a "bulged out" conformation, as proposed for purine-pyrimidine-purine sequences in ribonucleotides, where an increased stacking is observed between both purines and a decreased stacking between the purines and the pyrimidine.[12]

To assess more precisely the structural changes caused by *cis*-Pt in this trinucleotide, we are conducting a full conformational analysis of d(G-C-G)·*cis*-Pt.

Concluding, we have shown that as a result of *cis*-Pt binding, intrastrand cross-linking between two guanines separated by a third base is possible, at least at the trinucleotide level. Such a cross-link will produce a very different lesion in DNA in comparison with a cross-link via *cis*-Pt involving two adjacent guanines. This may be reflected in the mutation induction in *E. Coli* bacteria, where repair of lesions involving *cis*-Pt bound to two guanines separated by a third base apparently induces more base-pair substitutions.[7] Whether the described lesion plays a role in the mechanism of the antitumor action of *cis*-Pt remains to be investigated.

Acknowledgment. We are indebted to Professor Dr. J. H. van Boom for his kind gift of d(G-C-G) and to Dr. A. M. J. Fichtinger-Schepman for the platinum analyses. Professor Dr. R. Kaptein and K. Dijkstra are thanked for assistance at the 360-MHz (SON) NMR facility. This research was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Registry No. *cis*-Pt, 15663-27-1; d(G-C-G)Na, 81256-30-6; d(G-C-G)·*cis* Pt, 81277-04-5.

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Selectivity Profile of the Cation Radical Diels–Alder Reaction

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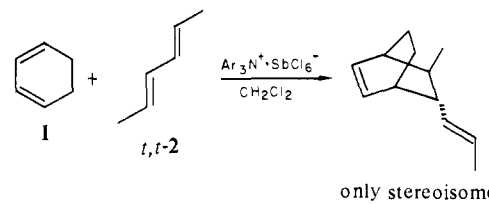
The recent observation of powerful catalysis of certain Diels–Alder cycloadditions by aminium cation radical salts[1] (the cation radical Diels–Alder, CRDA) makes conveniently available a number of Diels–Alder adduct structures that heretofore either were not directly available or were accessible in only poor to miniscule yields. In addition to the substantial intrinsic mechanistic and theoretical interest in this new reaction, it therefore appears to have potential for developing into a generally useful synthetic procedure. The suprafacial stereospecificity of the reaction, as established, for example, in the cycloaddition of 1,3-cyclohexadiene (**1**) to the three geometric isomers of 2,4-hexadiene (**2**) and illustrated in eq 1 for the trans, trans isomer, implies a



1 *t,t*-**2** only stereoisomer (I)

true pericyclic process analogous to the thermal (neutral) Diels–Alder. As examination of the orbital correlation diagram in Scheme I reveals, the prototype [4 + 1] cycloaddition (that between ethene cation radical and *s-cis*-1,3-butadiene to give

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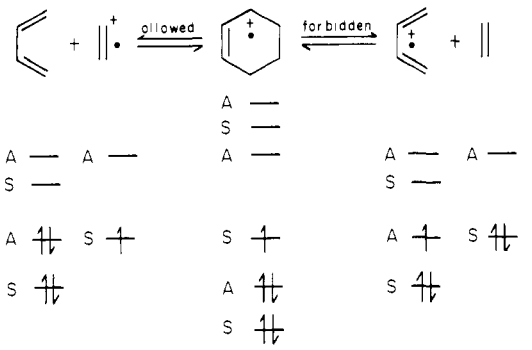
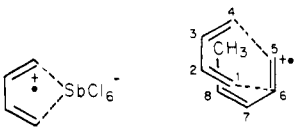
Scheme I



cyclohexene (π) cation radical) is allowed. Although allowed doublet pericyclic processes are novel they are not unprecedented, and the observation of a true pericyclic transition state for the CRDA is seen to be in full accord with theory.[2] In significant contrast, the [3 + 2] cycloaddition of neutral ethene to *s-cis*-1,3-butadiene cation radical to give cyclohexene cation radical is forbidden. The implicit preference of the cation radical for the dienophilic role has been observed experimentally[1,3] and termed "role selectivity". In effect, the cation radicals may be viewed as functioning as extremely electron deficient, and hence highly reactive, dienophiles.

The endo stereoselectivity found in the CRDA is equally impressive. In the example cited in eq 1 and in a considerable number of additional cases the endo isomer is obtained essentially exclusively (exo $\ll$ 1%). Typical endo stereoselection in the neutral Diels–Alder is quite modest; indeed, the uncatalyzed Diels–Alder reaction between **1** and *t,t*-**2** has somewhat surprisingly been found to yield an excess of the exo isomer (3:1; yield ca. 1%). The exclusive endo stereoselection found in many instances of the CRDA agrees well with the observation that increased electron deficiency in the dienophile engenders increased endo stereoselection in the neutral Diels–Alder. This trend is especially notable in the Lewis acid catalyzed neutral Diels–Alder.[4] The implication is again that the diene cation radicals function as highly electron-deficient dienophiles. Whereas exclusive endo stereoselection is typical in the CRDA of dienes (in the dienophilic role) that, like **2**, have no cis substituent on the exocyclic double bond (C_7–C_8 in **4**) the presence of such a substituent abruptly and grossly



3 4

attenuates the stereoselection (the "*cis*-propenyl effect"). In order to rationalize this conspicuous effect it appears necessary to postulate a preferred *s*-cis conformation for the diene cation radical. This could originate, for instance, in a chelate-like ion pairing effect (**3**) such as has previously been observed for diene anion radicals.[5] The long range ("secondary") interactions which are conventionally assumed to be the basis for endo stereoselection might then be enhanced by the interaction of not just one but both carbons (C_7–C_8 in **4**) of the exocyclic double bond with the endocyclic double bond (C_2–C_3) in the cation radical transition state. The *cis*-propenyl effect could then be attributed to inability to form the appropriate *s*-cis conformation and/or steric repulsions in the proposed endo,*s*-cis,*s*,cis transition state (**4**).

Equally as important in the total selectivity profile of the CRDA are the chemo- and regioselectivities. Chemoselection, in the present context, refers to the selection between chemically nonequivalent double bonds of an unsymmetric (dienophilic) diene. Regioselection refers to the orientational preference (e.g., *o*, *m*, *p*) involved when both reaction components are unsymmetric.

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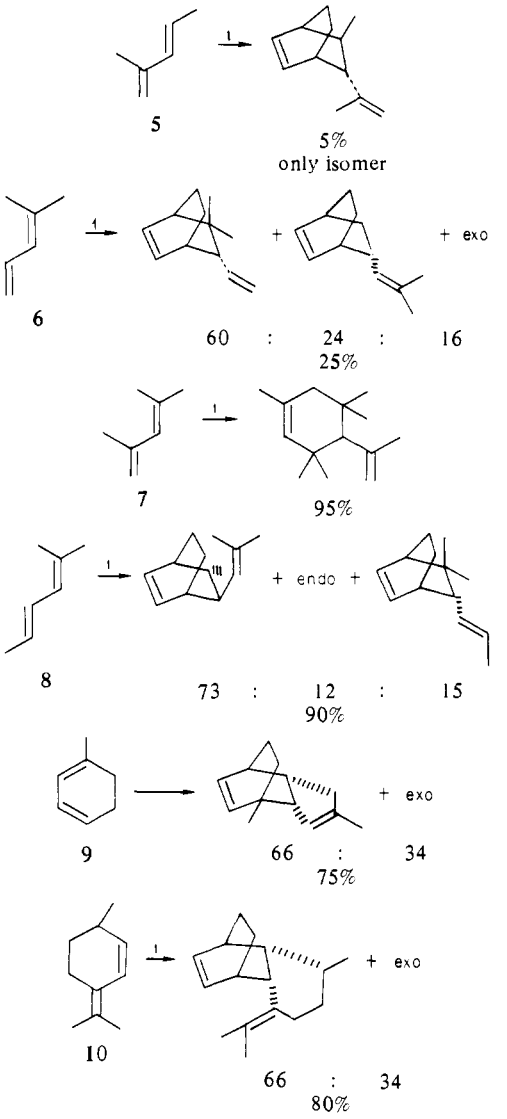
Scheme II

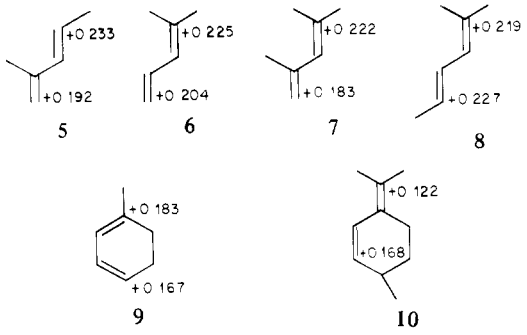


Knowledge of these final selectivity elements is essential to the evaluation of the CRDA for adoption in synthetic strategies. A basis set of six dienophiles (**5**–**10**) was selected for the study, which involved CRDA reactions of these dienophiles with 1,3-cyclohexadiene (**1**) or, in two cases, with themselves (dimerization). The new selectivities are no less impressive than the previous ones and are quite encouraging with respect to possible synthetic uses.

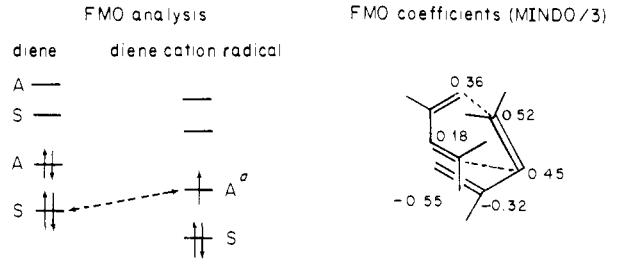
The new data in Scheme II reconfirm the stereospecificity and endo selectivity features (including the *cis*-propenyl effect) discussed earlier. In addition, it is noted that the yields tend to increase with alkyl substitution and are quite good when at least three alkyl substituents are present. These substituents, especially when present on the terminal diene carbons, stabilize the cation radical and tend to suppress the main competition of the CRDA, which is polymerization. Similarly, the more complex dienes also tend to react more selectively (both in chemo- and regioselectivity). Dienes **9** and **10** exhibit virtually exclusive chemo- and regioselection. It will also be noted that the acyclic diene **7** so far surpasses **1** in facility in the dienic role that only dimerization was observed in this case.

The chemoselection results contrast vividly with those expected in the neutral Diels–Alder reaction both in degree and sense and are empirically summarized as follows: Chemoselection favors that double bond which has the largest number of terminal alkyl substituents. A specific, but major exception is dienes with the (1,2) terminal substitution pattern (i.e., one terminal carbon having two and the other one alkyl substituent). The observed chemoselection patterns can be rationalized in terms of charge densities tempered by steric considerations in marginal cases (the (1,2)

Scheme III. MINDO/3 Charge Densities at Terminal Carbons of Diene Cation Radicals



Scheme IV



[a] Locally symmetric (i.e., bonding at C_5–C_6).

substitution pattern). MINDO/3 charge densities are displayed in Scheme III for the six dienophilic cation radicals. In every case but **9**, the charge density is greatest at the terminal carbon with the highest reactivity. In this specific case the charge differential is especially minute and may reasonably be presumed to be dominated by the substantial steric repulsions involved in the bond formation process at the disubstituted dienic terminus. The existence of a charge density correlation appears theoretically plausible for a reaction having an early (reactant-like) transition state and especially if the cycloaddition reaction path is nonsynchronous, as suggested by MINDO/3. An FMO analysis[6] of the pericyclic transition state leads to essentially the same chemoselection predictions and in addition permits prediction of regioselection (Scheme IV). The appropriate FMO analysis focuses on the *locally* symmetric dienophile cation radical SOMO and the most bonding diene π MO, which are the most proximate in energy. The relevant MINDO/3 coefficients are illustrated for **7** in Scheme IV. Once again, the chemoselectivities of (1,2) dienes **8–10** requires recognition of the predominance of steric effects in these marginal cases.

In exploiting the CRDA for synthetic purposes, it should be remembered that these reactions are usually complete in 10–15 min at 0 °C and longer reaction times can lead to yield losses. Consequently, GC or other monitoring is recommended. It has been found that 3–5% of the aminium salt, which is now available from Aldrich Chemical, is usually sufficient to assure rapid and complete reaction.

Present research is concentrating on extending the CRDA and establishing new types of cation radical pericyclic processes.

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Registry No. 3β-(2-Methylpropanol)[2.2.2]bicyclooct-5-ene, 40572-29-0; 2,2-dimethyl-3α-ethenyl[2.2.2]bicyclooct-5-ene, 81277-87-4; 2α-(2-methylpropenyl)[2.2.2]bicyclooct-5-ene, 40600-18-8; 2,4,4,6,6-pentamethyl-5-isopropylidenecyclohexene, 68930-33-6; 2α-methyl-3β-(2-methylpropenyl)[2.2.2]bicyclooct-5-ene, 81277-88-5; 2α-methyl-3α-(2-methylpropenyl)[2.2.2]bicyclooct-5-ene, 81339-57-3; 2,2-dimethyl-3α-propenyl[2.2.2]bicyclooct-5-ene, 81277-89-6; (1β,4β,4aβ,8aβ)-1,7-dimethyl-1,4,4a,5,6,8a-hexahydro-1,4-ethanonaphthalene, 81277-90-9; (1β,4β,4aα,8aα)-1,7-dimethyl-1,4,4a,5,6,8a-hexahydro-1,4-ethanonaphthalene, 81339-58-4; 5-methyl-1,4,4a,5,6,7,8a-heptahydro-8-isopropylidene-1,4-ethanonapthalene, 81277-91-0; **1**, 592-57-4; *t,t*-**2**, 5194-51-4; **3**, 81277-83-0; **5**, 1118-58-7; **5** radical cation, 74111-61-8; **6**, 926-56-7; **6** radical cation, 74056-42-1; **7**, 1000-86-8; **7** radical cation, 81277-84-1; **8**, 28823-41-8; **8** radical cation, 81339-55-1; 9, 1489-56-1; **9** radical cation, 81277-85-2; **10**, 586-63-0; **10** radical cation, 81339-56-2; 1,3-butadiene, 106-99-0; ethene radical cation, 34470-02-5; cyclohexene radical cation, 34469-90-4; 2β-methyl-3α-(1-methylethenyl)[2.2.2]bicyclooct-5-ene, 81277-86-3.

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Evidence by Gel Filtration at Subzero Temperatures for the Covalent Reaction Intermediate of Carboxypeptidase A in Ester Hydrolysis[1a]

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The primary advantage of cryoenzymologic methods[2-4] in the study of enzyme mechanisms derives from the potential to accumulate and stabilize reaction intermediates for structural and chemical characterization. With cryoenzymologic techniques, we have demonstrated temporal resolution of the esterolytic reaction catalyzed by carboxypeptidase A (CPA) and formation of a reaction intermediate that is best described as a mixed anhydride species.[5-7] X-ray diffraction studies[8,9] do not distinguish between the possible roles of the catalytically active residue glutamate-270 as a nucleophile in forming an acylenzyme (mixed anhydride) reaction intermediate or in positioning a hydrogen-bonded water molecule as the nucleophile in a general base mechanism. In addition, nucleophile trapping studies[10,11] designed to detect reaction intermediates of CPA have not been successful. In this communication we demonstrate that the reaction intermediate observed under subzero temperature conditions can be isolated by gel filtration at −60 °C and, therefore, is a covalent acylenzyme species.

The hydrolysis of the specific ester substrates *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactate[5,13] (ClCPL) and *O*-3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)propen-2-oyl-L-β-phenyllactate[14,15] (TEPOPL) catalyzed by CPA is governed by rate-limiting breakdown of the reaction intermediate that is detectable at subzero temperatures. The half-life of the reaction intermediate

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(11) Nucleophile trapping of a mixed-anhydride intermediate with, e.g., hydroxylamine, in principle is the most direct method to demonstrate a covalent reaction intermediate. In model reactions,[12] however, it has been shown that the interaction of the metal ion with the substrate renders this approach as unreliable. The results of recent EPR studies of the reaction intermediate of CPA formed during esterolysis show that the active-site metal ion coordinates the carbonyl oxygen of the scissile bond as well as a solvent molecule.[7] These factors are the likely origin for the lack of reproducible stoichiometries[12] in detecting reaction intermediates of CPA with hydroxylamine.
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