from the alkene and HCl. We believe that the tight ion pairs produced from the alkyl halides probably show relatively more recombination and that the rate-determining steps in the trifluoroethanolyses of *t*-butyl chloride and 1-phenylethyl chloride are also dissociation of the tight ion pairs.

Because of the absence of a technique to evaluate the importance of "hidden return" it has not heretofore generally been possible to make a distinction between two fundamentally different kinds of participation: namely (1) participation in the initial bond ionization and (2) participation in the process of further reaction of an initially formed ion pair.[16] The latter explanation is especially attractive for those examples where ion-pair return is known to be dominant in the reference compound and where the compound which appears to react *via* participation would give a carbonium ion which is subject to facile rearrangement to a more stable classical ion. Thus, since our experiments indicate that return of isopropyl cation–brosylate ion pairs in TFA is fast relative to solvolysis it is obvious that 3,3-dimethyl-2-butyl brosylate might ionize only slightly faster than isopropyl brosylate but have its solvolysis rate in TFA, relative to isopropyl, much accelerated if the Wagner–Meerwein rearrangement took place rapidly in the tight ion-pair stage; after rearrangement, return to the very reactive tertiary brosylate would not slow the rate and reverse rearrangement with return would be prohibited by the much higher energy of the secondary ion relative to the tertiary ion. Until the importance of tight ion-pair return can be evaluated it is unwarranted to accept rate acceleration as conclusive evidence for participation *in the first ionization step in reactions where facile rearrangement to a more stable classical ion is possible.*[17] An isotope effect in the migrating group only shows participation in the rate-determining step and does not serve to distinguish between the two types of participation.[18] Further, rate-determining proton loss from the tight ion pair could show a deuterium isotope effect similar to that associated with hydrogen participation.[19]

The reactions reported here were followed using a Varian HA-100 magnetic resonance instrument; characteristic peak positions in TFA solvent were as follows: isopropyl brosylate, δ 1.07 doublet, J = 7 Hz; isopropyl trifluoroacetate, δ 1.14 doublet, J = 7 Hz; isopropyl alcohol, δ 1.10 doublet, J = 7 Hz; propylene, δ 1.42, J = 1.7 and 7 Hz. The internal standard for these reactions was 1,4-dioxane. The chemical shift of dioxane is somewhat dependent on acid concentration.

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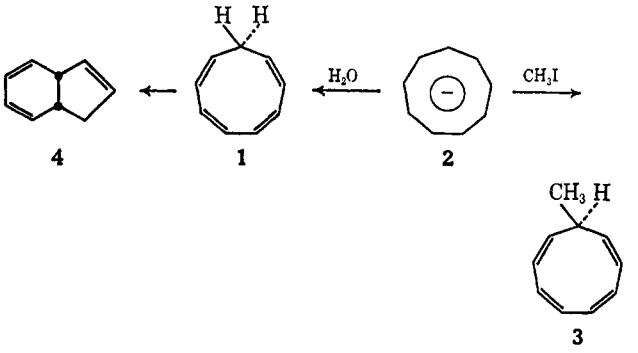
The Preparation and Isolation of *cis,cis,cis,cis*-1,3,5,7-Cyclononatetraene

Sir:

cis,cis,cis,cis-1,3,5,7-Cyclononatetraene (**1**) has been the object of much discussion in the literature but primarily since the successful preparation of cyclononatetraenide ion (**2**).[1-9] While the primary emphasis has been with regard to the intermediacy of **1** in thermal and photochemical transformations of other C_9H_{10} compounds, questions with regard to the stability, acidity, and general structural nature of **1** have also been raised. Despite the fact that there is some evidence[8] that it might be possible to isolate this very important C_9H_{10} olefin, there has been to date no report of its preparation and direct observation. It is the purpose of this communication to remedy this situation and preliminarily report the isolation of **1** and its 9-methyl derivative (**3**).



In a typical procedure a solution of **2** (20 mmoles) in tetrahydrofuran[7,8] (7 ml) at *ca.* 0° was quenched with ice water (20 ml) and was rapidly extracted into cold CCl_4 (9 ml). The cold CCl_4 extract was washed rapidly with ice-cold 1 *N* HCl followed by ice water. The cold extract was filtered through anhydrous $MgSO_4$, and an aliquot was used to obtain the nmr spectrum of **1** shown in Figure 1. The procedure for the 9-methyl derivative **3** was essentially the same except that CH_3I (20 mmoles) was added to the tetrahydrofuran solution of **2** at 0° and was allowed to react for 2.5 hr before the aqueous quench and work-up. Using this technique one can obtain solutions of **1** in the organic solvent of choice depending upon one's needs.

Solutions of **1** and **3** in ether were reduced at 0° with hydrogen and Raney nickel for *ca.* 6 hr. In the case of **1** about 60% cyclononane was obtained along with *cis*-hydrindan and in the case of **3** about 50% methylcyclononane[10] was produced along with the corresponding methyl-*cis*-hydrindans.[11]

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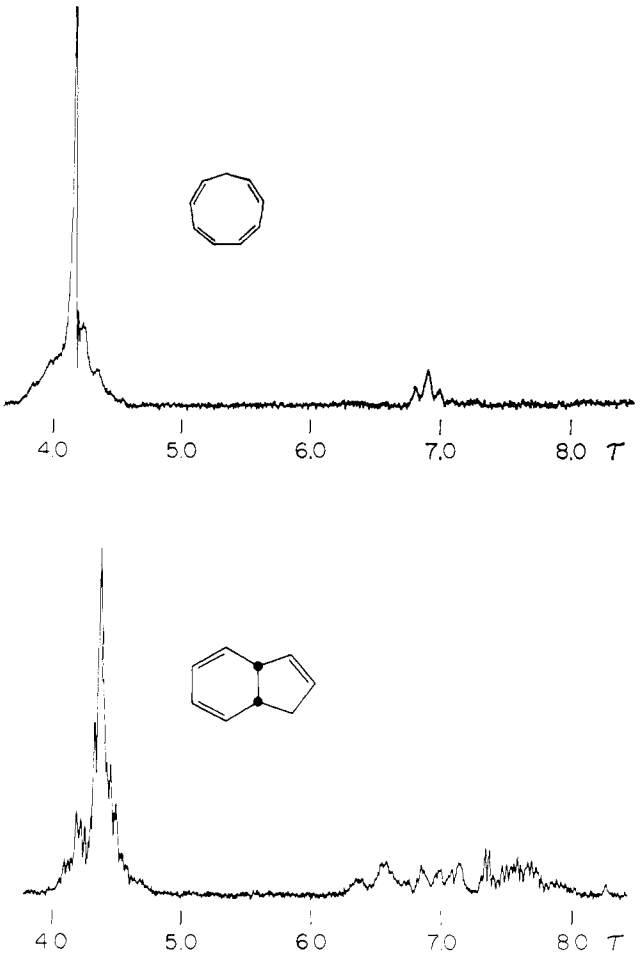

Figure 1. The 60-MHz nmr spectrum of **1** (top) and **4** (bottom) taken in CCl_4 at 38°.

The nmr spectrum of **1** is depicted in Figure 1 and shows the six vinyl protons at τ 4.15 and the two methylene protons at τ 6.88. Also shown in Figure 1 is the spectrum of the same sample taken after 5 hr at 40°. This spectrum is that of *cis*-8,9-dihydroindene (**4**). It is important to note here that no *trans*-8,9-dihydroindene is produced as a result of the thermal $6\pi \rightarrow 4\pi 2\sigma$ electrocyclic ring closure of **1**.[12] The nmr spectrum of **3** displayed a more complicated vinyl pattern for six protons at τ 4.22, a doublet for the methyl group at τ 8.88 (J = 7 Hz), and the tertiary proton at τ 8.70 (complex quartet). We have also taken infrared spectra of **1** and **3** in CCl_4 and find no absorption at 965 cm^{-1} characteristic of a *trans* double bond.

These data clearly indicate that we have isolated and observed directly **1** and **3**. The question now remains with regard to the degree of thermal stability of the *cis,cis,cis,cis*-1,3,5,7-cyclononatetraene skeleton. In qualitative experiments we have found that **1** has a half-life of *ca.* 14 min at 40°. A more quantitative result provided by Dr. Gary Petrowski[13] indicated that at 23° the rate constant for the thermal transformation of **1** → **4** is 6×10^{-5} sec^{-1}; the half-life for **1** at this temperature is 50 min.

Cyclononatetraene (**1**) now presents itself for further study as a remarkably stable compound. There are many questions which can now be raised and hopefully answered with respect to the properties and chemical reactivity of this very interesting olefin, and we hope to provide more information with regard to these points in the near future.

Acknowledgment. The authors gratefully acknowledge support from the Petroleum Research Fund of the American Chemical Society and partial support from the Intramural Research Fund of the University of California.

(12) One other compound is produced from **1** to the extent of about 7%; at present its structure is unknown.
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(15) National Science Foundation Undergraduate Research Fellow.

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α-Iminoglutarate Formation by Beef Liver L-Glutamate Dehydrogenase. Detection by Borohydride or Dithionite Reduction to Glutamate[1]

Sir:

The enzyme L-glutamate dehydrogenase catalyzes the reversible oxidative deamination of glutamate to α-ketoglutarate, with DPN or TPN as coenzyme.[2-4] The over-all reaction, in analogy with other oxidations of amines,[5-9] most likely proceeds through an intermediate α-iminoglutarate in at least two steps. Reaction 2

$$\text{glutamate} + \text{DPN} \rightleftharpoons \alpha\text{-iminoglutarate} + \text{DPNH} \quad (1)$$

$$\alpha\text{-iminoglutarate} + H_2O \rightleftharpoons \alpha\text{-ketoglutarate} + NH_3 \quad (2)$$

was at first thought to occur spontaneously,[2] but later studies have not shown evidence for the spontaneous formation of the postulated α-iminoglutarate.[3,4]

We wish to present here direct evidence that the reverse direction of reaction 2 is enzyme catalyzed. We have trapped the presumed α-iminoglutarate-^{14}C, formed from α-ketoglutarate-^{14}C and ammonia in the presence of glutamate dehydrogenase and absence of coenzyme, by reduction to glutamate-^{14}C with sodium borohydride or sodium dithionite. Representative results are shown in Table I.

The formation of glutamate was dependent on enzyme and α-ketoglutarate concentration. Controls, not shown, with omission of enzyme, α-ketoglutarate, or borohydride, or with enzyme inactivated by heat, 10^{-3} M $AgNO_3$, or 30% ethanol, or substitution of yeast alcohol dehydrogenase or bovine serum albumin for the enzyme, did not result in significant glutamate formation. The borohydride appeared to reduce the α-iminoglutarate bound to enzyme, since glutamate was not formed if borohydride was added before α-ketoglutarate or if nonlabeled α-ketoglutarate was added before

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