PTrMA-coated macroporous silica gel packing may be useful not only for the resolution of racemate but also for more general separation by liquid chromatography. The different chiral recognition ability of the soluble (+)-PTrMA-coated packing and the ground insoluble (+)-PTrMA packing is an important and interesting phenomenon from the viewpoint of not only resolution but also polymer chemistry. A further study on this is now in progress.

A variety of chiral polymers have been made,[18] but only a few of them have been used as effective packings in liquid chromatography.[19] The deriving force of chiral recognition in these resolutions is mainly polar interaction such as hydrogen bond. The PTrMA packings showed higher resolution in the reversed-phase system with methanol as eluant than in the normal-phase system with hexane.[2,4] Nonpolar interaction between the packing and a racemic compound appears to be a main factor in the chiral recognition in the present chromatography.

Acknowledgment. We thank Sumitomo Chemical Co. Ltd. for supplying us with the phenothrins used in this study. Y.O. gratefully acknowledges receipt of the Kurata Research Grant for financial support. A part of this work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan.

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[18] For review see: "Optically Active Polymers"; Selegny, E., Ed.; Reidel: Dodrrecht, 1979.

[19] For review, see: (a) Krull, I. S. *Adv. Chromatogr.* **1978**, *16*, 175. (b) Blaschke, G. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 13.

Solvation of Ion Paris. Solvation Complexes between the Ion-Paired 9-Fluorenone Anion Radical and Dipolar Aprotic Solvents

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Received April 14, 1981

Ethereal and more polar aprotic solvents are characterized by their strong cation and weak anion solvation power; therefore it has been naturally assumed that only cationic solvation by aprotic solvents can modify the structures of ion pairs and hence indirectly perturb the spin density distribution of the ion-paired anions. No report published so far describes the formation of solvation complexes between an ion-paired anion and aprotic solvents.

In this communication, using as solvents binary mixtures of toluene and dipolar aprotic solvents (dielectric constant > 15),[1] e.g., dimethyl sulfoxide (Me_2SO), *N,N*-dimethylacetamide (DMA), *N,N*-dimethylpropionamide (DMP), and hexamethylphosphoramide (HMPA), we present that the 9-fluorenone anion radical (Fl$^-$) complexes both a cation and a solvent molecule. In addition, the solvation processes of ion pairs in binary mixtures are described. The carbonyl ^{13}C hyperfine splitting of Fl$^-$,[2] which increases with an increase in cationic field acting on the carbonyl oxygen, can function as an ESR probe for studying the present solvation phenomena as it is sensitive to environmental factors.

The ion pair between Fl$^-$ and a sodium ion in toluene exists in the form of a diamagnetic ionic dimer or higher aggregates and shows a very weak and featureless ESR signal. Addition of small amounts of dipolar aprotic solvents (less than 10^{-2} mole fraction) gives rise to well-resolved hyperfine patterns. The ^{13}C splitting first decreases with an increase of the mole fraction of aprotic solvents and then approaches a limiting value (a_C) characteristic of each solvent when the mole fraction is 0.2–0.3 (Figure 1). a_C keeps constant until the ion pair dissociates into the free ions in a mole fraction of greater than 0.6 (the concentration of Fl$^-$ $\simeq$ 10^{-4} mol). a_C decreases in the order Me_2SO > *N,N*-dimethylformamide (DMF) > DMA > DMP > HMPA. This order is the decreasing order of Gutmann's acceptor number (AN), which expresses the strength of anion solvation power by solvents,[3] and also the ^{13}C splitting of the free Fl$^-$ decreases in this order.[4] These features could be explained according to reaction 1

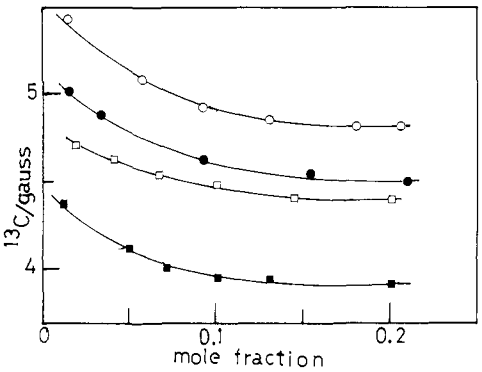

Figure 1. Change of the ^{13}C splitting by the additions of dipolar aprotic solvents to the toluene solution: (○) Me_2SO; (●) DMA; (□) DMP; (■) HMPA.

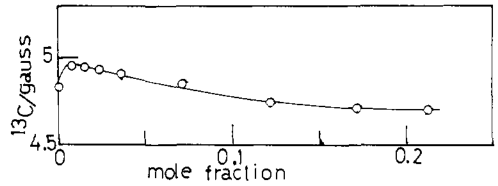

Figure 2. Change of the ^{13}C splitting by the addition of Me_2SO to the THF solution.

$$\underset{\text{(in toluene)}}{(RCO^-Na^+)_n} \rightleftarrows \underset{S}{RCO^-Na^+(S)_m} \rightleftarrows \underset{S}{RCO^-Na^+(S)_{m+1}} \quad (1)$$

where S is a dipolar aprotic solvent molecule. The solvation of the ion pair proceeds in two steps. In the first step, both the cation and the anion forming the ion pair are solvated by aprotic solvents. This step is responsible for decomposition of clustered ion pairs in toluene into monomeric ion pairs. The negative charge localized on the carbonyl oxygen attracts the positive end of a solvent dipole to form a solvation complex, and as a result, the oxygen atom is subject to additional cationic field. The solvation of the anion is completed at this step. With increasing fraction of aprotic solvents further solvation of the cation proceeds (the second step); this increases in solvation of the cation increases the cation–anion separation and reduces the ^{13}C splitting. At the end of the second step ^{13}C splitting approaches a_C. Except for HMPA a good linear relation between AN and a_C of each solvent was found (a similar linear relation between AN and ^{13}C splitting of the free Fl$^-$ was previously obtained).[4] This implies that the difference in a_C between the mixtures may almost entirely arise from the difference in anion solvation power between aprotic solvents. The value of a_C of each solvent is independent of temperature. Over a smaller range of mole fraction, on the other hand, the ^{13}C splitting decreases with decreasing temperature to converge to a_C at low temperatures. This indicates that the second step of reaction 1 is exothermic, and on lowering the temperature the equilibrium shifts to the direction of increasing solvation of the cation. After completion of the solvation shell, the ^{13}C splitting becomes independent of temperature. Thus, the dependences of the ^{13}C splitting both on temperature and on mole fraction of aprotic solvents can be interpreted in terms of the same equilibrium between solvation complexes.

Another evidence for the complex formation between the ion-paired anion and dipolar aprotic solvents was obtained by the

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[1] A. J. Parker, *Q. Rev., Chem. Soc.*, **16**, 163 (1962).

[2] 9-Fluorenone containing 90.9% enriched carbonyl ^{13}C was used.

[3] (a) V. Gutmann, *Struct. Bonding (Berlin)*, **12**, 113 (1973); (b) *ibid.*, **15**, 141 (1973); (c) *Electrochim. Acta*, **21**, 661 (1976).

[4] K. Nakamura, *Chem. Lett.*, 301 (1980).

addition of Me_2SO to the tetrahydrofuran (THF) solution of Fl^-Na^+. In the mixture of Me_2SO and THF, the ^{13}C splitting has a maximum at 0.005–0.006 mole fraction of Me_2SO and approaches a limiting value with a mole fraction of greater than 0.2 (Figure 2). The existence of the maximum indicates that there are two processes; one is the process which increases the ^{13}C splitting and the other decreases the ^{13}C splitting; first the former takes place. Reaction 2 depicts the two processes

$$\underset{\text{(in THF)}}{\mathrm{RCO^-Na^+(T)}_n} \rightleftarrows \underset{\mathrm{D}}{\mathrm{RCO^-Na^+(T)}_n} \rightleftarrows \underset{\mathrm{D}}{\mathrm{RCO^-Na^+(T)}_{n-1}\mathrm{(D)}} \quad (2)$$

where T is a THF and D a Me_2SO molecule. In the first step the ^{13}C splitting is increased by the solvation of the anion while the second step increases the anion–cation separation to reduce ^{13}C splitting.[5] The solvation of the anion easily takes place since the anion is nearly naked in THF whereas the approach of a Me_2SO molecule to the sodium ion is hindered by the already solvating THF molecules.

In a comparison of the mixtures of toluene and dipolar aprotic solvents, the mixture of toluene and THF exhibits several characteristic features: (1) The decomposition of the clustered ion pairs into monomeric ones occurs only when a considerable amount of THF is added (in less than 0.4 mole fraction of THF, the ESR signal is weak and featureless). (2) In any mole fraction of THF, the ^{13}C splitting does not approach a limiting value but monotonously decreases until the fraction of THF becomes unity (similar features were observed with the mixture of toluene and pyridine). The ^{13}C splitting of the pure THF solution of Fl^-Na^+ is known to be appreciably temperature dependent.[6] These facts suggest that even in pure THF, the solvent shell around the Na^+ forming the ion pair does not get completed, but two different solvation states exist in equilibrium.

Every ESR spectrum of the Fl^-Na^+ ion pair studied exhibits sodium hyperfine lines. This indicated that in any solvation stage the Fl^-Na^+ ion pair cannot exist in the form of a solvent separated ion pair, but the contact Fl^-Na^+ ion pair directly dissociates into the free ions.

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(5) K. S. Chen, S. W. Mao, K. Nakamura, and N. Hirota, *J. Am. Chem. Soc.*, **93**, 6004 (1971). In this paper, mixtures of ethereal solvents and DMF were used, and only the second step was assumed.

(6) N. Hirota, *J. Am. Chem. Soc.*, **89**, 32 (1967).

24(*S*),25-Epoxycholesterol Is a Natural Product of Mammalian Steroid Biosynthesis

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Received August 21, 1981

We have recently shown[1] that squalene 2,3(*S*);22(*S*),23-dioxide (**1**) is converted, upon incubation with S_{10} rat liver homogenate (RLH),[2] to the previously uncharacterized 24(*S*),25-epoxycholesterol (**2**),[3] with an efficiency comparable to that of the conversion of squalene 2,3(*S*)-oxide to cholesterol.[4] This result was obtained by use of biosynthesized **1**, which accumulates along with squalene oxide when mevalonate is incubated with RLH in the presence of 4,4,10β-trimethyl-*trans*-decal-3β-ol (TMD),[1] an effective inhibitor of oxidosqualene cyclase (EC 5.4.99.7).[5,6] We now report that 24(*S*),25-epoxycholesterol (**2**) is biosynthesized in RLH in the absence of inhibitor.

The search for **2** as a natural product of mammalian steroid biosynthesis was triggered by observation of a small amount of material with the TLC mobility[7] of squalene dioxide **1** in the nonsaponifiable extract[2] from RLH incubations to which no cyclase inhibitor had been added. Because our previous work[1] convinced us that if **1** were indeed present, the more stable, crystalline **2** would inevitably also be formed, we undertook to determine if the steroids produced in RLH contained **2**.

Incubations of [^{14}C]acetate[8] with RLH in the standard manner[2] afforded a nonsaponifiable extract[2] which had incorporated 2.0% of the initial radioactivity. TLC analysis[7] showed that this ^{14}C was divided among three major bands with the R_f values of squalene (18%), lanosterol (15%), and cholesterol (66%). Because we knew that 24(*S*),25-epoxycholesterol (**2**) migrated essentially the same as cholesterol upon TLC,[1] attention was focused on the cholesterol fraction (CF),[9] which contained 65% of the originally isolated ^{14}C after separation by preparative TLC.[7]

The first indication that the CF might contain **2** was obtained by treatment of a portion of it with $HClO_4$ in H_2O/THF.[10] Analysis by TLC of the product from this procedure showed 74% of the ^{14}C with unchanged R_f and 10% of the ^{14}C as much more polar material. When authentic **2**[3] was treated with $HClO_4$ in the same manner, essentially all the product had the same R_f as the more polar product. These observations suggested that there was [^{14}C]**2** in the CF, which, like the authentic unlabeled **2**, had undergone conversion, probably to a triol,[11] upon treatment with aqueous acid.

Confirmation that **2** was indeed present was obtained by benzoylation of a portion of the CF,[12] dilution of the product with authentic 24(*S*),25-epoxycholesterol benzoate,[1,10] purification by preparative TLC, and recrystallization to constant specific activity.[13] The final radioactivity obtained in the purified benzoate of **2** was 1.2% of that in the CF. However, the earlier results from the $HClO_4$ treatment had suggested that there was a substantially greater amount of **2** present. Since it was plausible that we were not isolating all of the **2** as its benzoate derivative,[14] we decided also to employ $LiAlH_4$ reduction of the CF in order to convert **2** to 25-hydroxycholesterol (**3**), which is stable and easily separable from cholesterol.[1]

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(1) Nelson, J. A.; Steckbeck, S. R.; Spencer, T. A. *J. Biol. Chem.* **1981**, *256*, 1067–1068.

(2) Popjak, G. *Methods Enzymol.* **1969**, *15*, 438–440.

(3) We have obtained **2**, mp 160–162 °C, and its 24(*R*) epimer, mp 166.5–168 °C, from their known[10] benzoate derivatives by saponification and have fully characterized both compounds.

(4) Corey, E. J.; Russey, W. E.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1966**, *88*, 4750–4751. van Tamelen, E. E.; Willett, J. D.; Clayton, R. B.; Lord, K. E. *Ibid.* **1966**, *88*, 4752–4754.

(5) Nelson, J. A.; Czarny, M. R.; Spencer, T. A.; Limanek, J. S.; McCrae, K. R.; Chang, T. Y. *J. Am. Chem. Soc.* **1978**, *100*, 4900–4902.

(6) Chang, T. Y.; Schiavoni, E. S., Jr.; McCrae, K. R.; Nelson, J. A.; Spencer, T. A. *J. Biol. Chem.* **1979**, *254*, 11258–11263.

(7) TLC analyses were performed on LK5D silica gel plates (Whatman, Inc., Clifton, NJ); preparative TLC plates were prepared with Silica Gel 60 PF-254+366 (EM Laboratories, Inc., Elmsford, NY). Various ratios of ether–hexane were employed as eluent.

(8) Sodium [1-^{14}C]acetate, specific activity = 56.7 mCi/mmol, was purchased from Amersham Corp, Arlington Heights, IL.

(9) A study of the composition of the CF obtained from an incubation of [^{3}H]lanosterol (**4**) with RLH by GLC (Varian Aerograph Model 2100, using 3% OV-17 on Gas-Chrom Q, Applied Sciences Laboratories, Inc., State College, PA, on a 6-ft. × $^1/_8$-in. glass column at 240 °C) showed 80% of the ^3H as cholesterol and 15% of the ^3H as a yet unidentified component. 24-(*S*),25-epoxycholesterol (**2**) decomposes under these GLC conditions. We are continuing to try to develop a GLC or HPLC method for the direct detection of **2** in the CF.

(10) According to the conditions of Seki et al. (Seki, M.; Koizumi, N.; Morisaki, M.; Ikekawa, N. *Tetrahedron Lett.* **1975**, 15–18) for the conversion of 24(*R*)- and 24(*S*),25-epoxycholesterol benzoates to 24,25-dihydroxycholesterol benzoates.

(11) Both 24,25-dihydroxycholesterols have been prepared by: Partridge, J. J.; Toome, V.; Uskokovic, M. R. *J. Am. Chem. Soc.* **1976**, *98*, 3739–3741. We did not attempt to characterize the polar product from the CF or authentic **2**.

(12) Typical benzoylation procedure: To 100 μg of alcohol in 100 μL of PhH was added 3 μL of pyridine and 2 μL of benzoyl chloride; the mixture was stirred at room temperature for 4 h and evaporated to dryness under vacuum.

(13) Initial specific activity = 480 dpm/mg. Recrystallization from acetone yielded **2**-benzoate with, successively, 250, 200, 160, 130, 130, and 120 dpm/mg.

(14) Possible reasons why the yield of pure **2**-benzoate may have been low include: incomplete benzoylation, decomposition of **2**-benzoate during purification, and difficulty in separation from cholesterol benzoate.

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