# Adsorption of Amino Acid Derivatives by *d*- and *l*-Quartz

**Percy R. Kavasmaneck and William A. Bonner*[1]**

*Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305.* *Received May 19, 1976*

**Abstract:** The adsorption by quartz of alanine derivatives blocked at the COOH group by esterification (**2**), at the $NH_2$ group by acylation (**3**), and at both groups (**4**) was studied to see which group was primarily responsible for the adsorption. It was found that the $NH_2$ (or $NH_3^+$) group had to be free for effective adsorption to occur. The comparative adsorption of a number of amino *n*-butyl ester hydrochlorides was investigated to see what type of esterified amino acid was most strongly adsorbed. Basic amino esters were adsorbed most effectively, hydroxylic and neutral amino esters next, and esters of acidic amino acids least. The adsorption of *n*-butyl ester hydrochlorides of each type of amino acid varied inversely with molecular weight. The adsorption of alanine isopropyl ester hydrochloride (**2**) by quartz was most pronounced from nonpolar solvents, and decreased as the polarity of the solvent increased. The adsorption of **2** from chloroform solution was studied as a function of concentration, and the resulting adsorption isotherm was found to be clearly of the Langmuir type. Finally, the asymmetric adsorption of (*R,S*)-**2** by *d*- and *l*-quartz was studied by fractional elution, using gas chromatography for the analytical estimation of enantiomeric enrichment. *l*-Quartz preferentially adsorbed (*R*)-**2** and *d*-quartz (*S*)-**2**, the extent of enantiomeric enrichment among the various fractions varying between 1.5 and 12.4%.

The question of the origin of optically active organic molecules in nature has intrigued scientists since the time of Pasteur, since such molecules are intimately associated with the existence of life on earth. Hypotheses and mechanisms proposed since Pasteur's time for the origin of optical activity have been recently reviewed.[2-5] Since many of the pertinent experimental observations were frequently quite marginal, however, we have more recently been interested in reinvestigating some of the proposed abiotic mechanisms experimentally, using modern instrumental techniques.

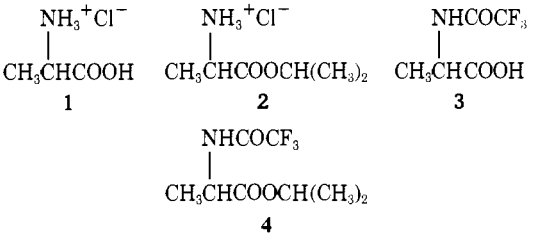
Over 40 years ago, Tsuchida and co-workers[6] reported the partial resolution of certain racemic cobalt complexes on chromatographic columns packed with *d*- or *l*-quartz powder. Shortly thereafter, Karagunis and Coumoulos[7] extended the technique to the resolution of a chromium complex and thereupon suggested that such asymmetric adsorption on the surfaces of optically active minerals might have engendered the first optically active organic molecules in nature. This suggestion, later amplified by Bernal,[8] appeared to receive abundant (if marginal) confirmation in succeeding years, when the asymmetric adsorption or partial resolution of many additional racemic inorganic complexes with *d*- or *l*-quartz were reported (cf. Bonner[3]), as well as those of 2-butanol and active amyl alcohol.[9]

In 1968, however, Amariglio and co-workers[10] cast serious experimental doubt on the dozen or so partial resolutions with quartz which had been reported in the earlier literature. They attempted to duplicate four of the previously reported resolutions, employing a very sensitive photoelectric polarimeter to measure any resulting optical activity, and being particularly careful to exclude artifacts (due to residual suspended quartz dust) during optical rotation measurements. No optical rotation beyond experimental error was noted in the eluate fractions from any of their experiments, however, one of which was even conducted at dry ice temperature. Amariglio et al.[10] thereupon undertook a detailed critical analysis of all previous "successful" quartz resolutions and, enumerating the many experimental pitfalls which could lead to spurious positive results, concluded that these earlier reports were uniformly erroneous.

Because of these contradictions in the literature and the possible importance of asymmetric adsorption as a mechanism for the origin of optical activity, we have recently undertaken to reinvestigate the question of asymmetric adsorption by quartz. Due to the low optical rotations anticipated in such experiments, however, we chose to estimate asymmetric adsorption by techniques not depending upon direct polarimetric measurements. Furthermore, we elected to employ amino acids as adsorbates, since such previously uninvestigated substrates would be more pertinent and realistic from the viewpoint of prebiotic chemical evolution. Our initial experiments[11] employed the loss of radioactivity from solutions of $^{14}C$- and $^{3}H$-labeled (*R*)- or (*S*)-alanine hydrochloride after exposure to powdered quartz as a criterion for gross adsorption and the difference in adsorption by *d*- vs. *l*-quartz as a measure of asymmetric (differential) adsorption. These experiments showed that (*R*)- and (*S*)-alanine hydrochloride in $10^{-5}$ M dimethylformamide (DMF) solutions were adsorbed by *d*- or *l*-quartz to the extent of 20–30% and that *d*-quartz preferentially adsorbed (*R*)-alanine and *l*-quartz (*S*)-alanine hydrochlorides, the differential adsorption being 1–1.8%. More recently we have confirmed and amplified these observations[12] using as adsorbate radioactive (*R,S*)-alanine hydrochloride having either the (*R*)- or (*S*)-enantiomer separately labeled. In these experiments, the differential adsorption (defined somewhat differently) ranged from 12 to 20%. In view of these successful preliminary results, we thought it desirable to investigate more intensively the gross and asymmetric adsorption of other amino acid derivatives by quartz, both from the viewpoint of certain mechanistic details as well as the application of another experimental technique independent of optical rotation, namely, gas chromatography (GC). These investigations are the subject of the present communication.

## Results and Discussion

**The Role of Amino and Carboxyl Groups in Adsorption.** In our original experiments[11,12] involving (*R,S*)-alanine hydrochloride (**1**) as adsorbate, it was not possible to determine



whether the $NH_3^+$ group, the COOH group, or both groups interacted with the quartz surface to produce the asymmetric

Table I. The Adsorption of (*R,S*)-Alanine-3-$^3$H Derivatives by *l*-Quartz[a]

| No. | Derivative | Radioactivity counts[b] Original stock solution (*A*) | Supernatant after adsorption (*B*) | Adsorption, %[c] |
|---|---|---|---|---|
| 1 | Isopropyl ester hydrochloride, **2** ($NH_3^+$ free) | 78 519 | 7 656 | 90.2[d] |
| 2 | *N*-Trifluoroacetyl, **3** (COOH free) | 57 221 | 47 562 | 16.9 |
| 3 | *N*-Trifluoroacetyl isopropyl ester, **4** | 77 300 | 77 805 | (0.0) |

[a] 5 g; particle size <45 μm. [b] Counts per 2 min for 300-μl aliquot. [c] $100(A - B)/A$. [d] Adsorbed material recovered in >90% yield by washing quartz with 10 ml 1 N HCl.

adsorption noted. If such a determination were possible, the interpretation of the asymmetric adsorption process might be simplified and a derivative might be designed which would enhance the asymmetric bias. To attempt this determination, we have prepared derivatives of (*R,S*)-alanine-$^3$H where the $NH_2$ and COOH groups were selectively blocked and then have studied the gross adsorption of each derivative by *l*-quartz using anhydrous dichloromethane (DCM) as solvent. As before, the gross adsorption was determined by comparing the radioactivity level of the supernatant solution after exposure to the quartz with that of the original DCM solution. The radioactivity and adsorption data are summarized in Table I. We see that blocking the COOH group while leaving the $NH_3^+$ function free ((*R,S*)-alanine isopropyl ester hydrochloride, **2**) gives a derivative capable of being adsorbed to over 90% by *l*-quartz under the conditions employed. Blocking the $NH_3^+$ group while the COOH remains free (*N*-TFA-(*R,S*)-alanine, **3**), on the other hand, permits less than 17% gross adsorption, while blocking both groups (*N*-TFA-(*R,S*)-alanine isopropyl ester, **4**) renders the amino acid derivative incapable of being adsorbed at all. The high adsorption shown by **2** was confirmed by decanting the DCM supernatant and extracting the quartz residue with dilute HCl, whereupon over 90% of the original radioactivity was recovered. These experiments clearly show that the $NH_3^+$ (or $NH_2$) group of the amino acid must be free for really effective adsorption by quartz to be possible.

**Comparative Adsorption of Amino Acid *n*-Butyl Ester Hydrochlorides.** Having established that an amino acid ester hydrochloride showed more adsorption by quartz than did other derivatives studied, we next sought to establish which amino ester would be most suitable for subsequent asymmetric adsorption studies. This was done by dividing ten different amino acid *n*-butyl esters into three sets of mixtures, each of which could be conveniently and quantitatively analyzed by GC after conversion to its *N*-trifluoroacetyl derivative mixture. Each *n*-butyl ester set (containing one radioactive ester component to serve as an internal standard for the estimation of gross adsorption) was dissolved in DCM, and a portion of the resulting stock solution was stirred with *d*-quartz powder in the usual way. Gross adsorption of the *labeled* component in each set was determined as before by radioactivity count loss of the supernatant as compared with the original stock solution. Each stock solution and corresponding supernatant was then trifluoroacetylated and the resulting mixtures were analyzed by GC. The "corrected" GC integration count for each component of each supernatant, as calculated via the labeled internal standard of each set, was compared with the corresponding count for that component of each stock solution, thus permitting calculation of the percent gross adsorption of each component in each set. The comparative adsorption data so obtained are shown in Table II.

Examination of Table II allows several qualitative conclusions regarding the adsorption of amino acid *n*-butyl ester hydrochlorides by quartz. We see (last column) that the effectiveness of quartz in adsorbing such esters is greatest for the basic amino acids (Lys, Try), less for a hydroxyl-containing neutral amino acid (Thr), still less for neutral amino acids (Ala, Val, Leu, Pro, Phe), and least for acidic amino acids (Glu, Asp). It is thus clear that the adsorption by quartz of amino *n*-butyl ester hydrochlorides in DCM is strongly dictated by whether the ester is derived from a basic, neutral, hydroxylated, or acidic amino acid. Furthermore, within each class of amino acid the extent of adsorption is influenced by the size of the adsorbate molecule, varying inversely with molecular weight (except in the case of proline) within each class.

Attempts to use a basic amino ester (lysine *n*-butyl ester hydrochloride) in asymmetric adsorption experiments, with the hope of capitalizing on its greater adsorption capability, proved futile, since gas chromatography of the *N*-TFA-(*R*)- and (*S*)-lysine-(+)-2-butyl (or isopropyl) ester derivatives subsequently prepared for enantiomer ratio analysis resulted in only partial resolution of the diastereomer (or enantiomer) mixtures. Since the corresponding derivative of (*R,S*)-threonine also appeared unpromising (being unstable, like similar hydroxy amino acid derivatives),[13] an ester of the "most promising" neutral amino acid, namely, alanine isopropyl ester hydrochloride, **2**, was chosen for subsequent adsorption studies.

**Adsorption from Different Solvents.** In this aspect of our study, a solvent system was sought which would permit substantial, but not complete, adsorption of **2** from ca. $10^{-4}$ M solution by quartz and at the same time would allow convenient solvent removal for subsequent derivatization and GC analysis of the residual adsorbate. Several aprotic solvents, as well as several binary mixtures, were studied from the viewpoint of the gross adsorption they permitted, again using radioactivity count loss as a criterion for adsorption. The results of these experiments are summarized in Table III.

The first set of experiments in Table III indicates that adsorption of the amino ester hydrochloride by quartz is greatest in a nonpolar solvent ($C_6H_6$) and decreases as the polarity of the solvent increases ($CH_2Cl_2$ and $CHCl_3$). The same thing is evident in the binary mixture experiments in Table III. Here the presence of a mere few percent of a protic ($CH_3OH$) or highly polar ($HCONMe_2$; DMF) solvent in $CH_2Cl_2$ decreases the adsorption from over 90% to essentially zero. This trend is less evident in the adsorption noted using binary mixtures of $CH_2Cl_2$ and $CHCl_3$, where increasing amounts of the more weakly polar $CHCl_3$ cause only a very gradual decrease in the gross adsorption. The lack of adsorption of the amino ester hydrochloride in a solvent mixture containing DMF is in sharp contrast to the significant adsorption from DMF previously noted[11,12] for unesterified alanine hydrochloride. The overall conclusions from Table III are that more polar solvents lead to less gross adsorption, that less polar solvents permit more gross adsorption, and that chloroform appears to be the most suitable solvent of those studied for attaining an intermediate extent of adsorption.

**Adsorption as a Function of Concentration and Temperature.** With the objectives of estimating the adsorption capacity of quartz and the fraction of its surface covered during adsorption, a series of experiments was conducted wherein chloroform solutions of radioactive (*S*)-**2** varying in concentration between $5 \times 10^{-5}$ and $10^{-3}$ M were exposed to *l*-quartz, and the gross adsorption at each concentration was measured by radioactivity count loss in the usual way. The data obtained are pre-

**Table II.** Comparative Adsorption of Amino *n*-Butyl Ester Hydrochlorides by Quartz

| | | GC analytical data[a-c] | | | |
|---|---|---|---|---|---|
| | | | Integration counts | | |
| Set | Amino ester HCl | Supernatant, rel %[g] | Stock (*A*) | Supernatant (*B*) ("corrected") | % adsorption[j,k] |
| I[a] | Ala-$^{14}$C[d] | 9.64 | 3 044 | 803[h] | 73.6 ± 2.8 |
| | Val | 26.08 | 4 158 | 2 172[i] | 47.8 ± 0.5 |
| | Leu | 31.50 | 4 850 | 2 624 | 45.9 ± 1.45 |
| | Pro | 32.78 | 4 168 | 2 730 | 34.6 ± 0.9 |
| II[b] | Try | 3.52 | 5 471 | 743 | 86.4 ± 2.6 |
| | Glu-$^{14}$C[e] | 28.86 | 10 664 | 6 091[h] | 42.9 ± 1.1 |
| | Phe | 34.19 | 12 110 | 7 216 | 40.4 ± 1.1 |
| | Asp | 33.43 | 9 830 | 7 055 | 28.2 ± 2.2 |
| III[c] | Lys | 1.41 | 10 129 | 766 | 92.4 ± 1.6 |
| | Try | 5.19 | 4 719 | 2 826 | 40.1 ± 3.7 |
| | Thr | 10.61 | 8 323 | 5 784 | 30.5 ± 2.7 |
| | Ala-$^{3}$H[f] | 10.47 | 6 877 | 5 708[h] | 17.0 ± 0.4 |
| | Pro | 15.58 | 9 490 | 8 490 | 10.5 ± 3.3 |
| | Leu | 17.49 | 10 473 | 9 528 | 9.0 ± 2.5 |
| | Glu | 20.09 | 11 985 | 10 947 | 8.7 ± 1.4 |
| | Asp | 19.16 | 11 398 | 10 442 | 8.4 ± 1.3 |

[a] 90 °C isothermally for Ala, Val; 140 °C isothermally for Leu, Pro; He flow, 9 ml/min. [b] 140 °C isothermally for Asp, Phe; 140-220 °C at 8 °C/min for Glu, Try; He flow, 9 ml/min. [c] 90-220 °C at 8 °C/min; He flow, 9 ml/min. [d] Radioactivity counts: stock, 95 206 ± 817; supernatant, 25 105 ± 404. Percent adsorption, 73.63 ± 2.85. [e] Radioactivity counts: stock, 35 023 ± 281; supernatant, 20 005 ± 209. Percent adsorption, 42.88 ± 1.06. [f] Radioactivity counts: stock, 289 195 ± 735; supernatant, 240 012 ± 954. Percent adsorption, 17.00 ± 0.40. [g] As determined by integration of GC peaks. [h] Actual GC integration count for labeled internal standard. [i] "Corrected" integration count calculated from *h* as: 26.08 × 803/9.64 = 2172. Other "corrected" counts calculated in a similar way. [j] Calculated as 100 $(A - B)/A$. [k] All (±) values in this and subsequent tables represent standard deviations.

**Table III.** Effect of Solvent on the Adsorption of Alanine Isopropyl Ester Hydrochloride by Quartz

| Solvent system | % adsorption[a] | | | | |
|---|---|---|---|---|---|
| Pure solvents[b] | | | | | |
| $CHCl_3$ | 45.2 | | | | |
| $CH_2Cl_2$ | 66.3 | | | | |
| $C_6H_6$ | 95.2 | | | | |
| | % adsorption with % $CH_2Cl_2$ in mixture = | | | | |
| | 100 | 98 | 90 | 50 | 0 |
| Binary mixtures | | | | | |
| $CH_2Cl_2$-$CH_3OH$[c] | 93.8[e] | 6.2 | 0 | — | 0 |
| $CH_2Cl_2$-DMF[c] | 90.6 | 0 | 0 | — | 0 |
| $CH_2Cl_2$-$CHCl_3$[d] | 84.9 | — | 82.8 | 75.2 | 57.0 |

[a] From 2 ml of $10^{-4}$ M solution. [b] Using 1.5 g quartz. [c] Using 2.0 g quartz. [d] Using 1.8 g quartz. [e] 89.8% of this adsorbed sample was recovered by rinsing the residual quartz with 2 ml of a 2% solution of $CH_3OH$ in $CH_2Cl_2$.

sented in Table IV, where the gross adsorption is seen to vary between 11% in the most concentrated to over 93 in the most dilute solution. The actual weight ($X$) of amino ester hydrochloride adsorbed per gram of quartz from each solution is readily calculated from the concentration and percent adsorption data and is seen to reach a saturation value of ca. 37 μg/g (corresponding to 0.22 μmol/g) for concentrations above $5 \times 10^{-4}$ M.

A plot of the weight per gram adsorbed ($X$) vs. (*S*)-**2** concentration ($C$) in Table IV shows that, despite the limited number of data points available, the isotherm obtained is empirically of the Langmuir form:[14]

$$X = \alpha C/(1 + \beta C) \quad (1)$$

The constants $\alpha$ and $\beta$ in (1) can be evaluated by rearranging (1) into the linear forms (2) or (3),

$$C/X = 1/\alpha + \beta C/\alpha \quad (2)$$

$$X/C = \alpha - \beta X \quad (3)$$

then plotting $C/X$ vs. $C$ (Figure 1b) or $X/C$ vs. $X$ (Figure 1c), respectively. The average values of the constants, obtained by least-squares fitting of the data to both linear eq 2 and 3 are: $\alpha$ = 512 μg/g/mmol/l. and $\beta$ = 12.5 l./mmol. The success of eq 1 and 2 in describing the adsorption isotherm for (*S*)-**2** in chloroform by quartz is also seen in Table IV, where the observed values for $X$ and $C/X$ are compared with those calculated by (1) and (2), respectively, using the indicated average values for $\alpha$ and $\beta$.

A useful application of the Langmuir adsorption isotherm is the estimation of the maximum adsorption capacity of the adsorbent, $X_0$. Thus as $C \rightarrow \infty$ (i.e., $X/C \rightarrow 0$) in eq 3, $X \rightarrow X_0$. This maximum adsorption ($X$ axis intercept in Figure 1c) has a value of ca. 41.0 μg/g in the present experiments, a value which permits estimation of the fractional surface coverages at the lower concentration seen in Table IV. The surface coverage ranges from a little over one-third at the lowest concentration to over 90% at saturation.

If the assumption is made that the effective cross-sectional area of the **2** molecule under conditions of maximum adsorption (when $X = X_0$) is equal to the specific area ($A_0$) when such molecules are spread as a unimolecular film at an air-water interface,[15] a rough estimate of the surface area of the quartz sample can be made. Knowing the value of $X_0$ in moles of adsorbate per gram of quartz (ca. $2.5 \times 10^{-7}$) and assuming the limiting area ($A_0$) of the adsorbed molecule to be 25 Å$^2$,[15] the product of $X_0$, $A_0$, and Avogadro's number gives 0.037 m$^2$/g as the estimated surface area of the present quartz sample. This figure agrees well with the surface area, 0.025 m$^2$/g, calculated geometrically for nonporous quartz spheres of 90 μm average diameter and 2.65 g/cm$^3$ density.[16] Both of these values are

Table IV. Adsorption Isotherm (25 °C) for (*S*)-Alanine-$^3$H Isopropyl Ester Hydrochloride in Chloroform by *l*-Quartz[a]

| Concn, *C*, M × $10^4$ | Radioactivity counts: Stock (*A*) | Radioactivity counts: Supernatant (*B*) | % [b] | Adsorption: *X*, μg/g Found | *X*, μg/g Calcd [c] | *C*/*X*, M × $10^3$/μg Found | *C*/*X*, M × $10^3$/μg Calcd [d] | $X_m$, μmol/g | Surface coverage: % [e] | Surface coverage: Molecules/cm² × $10^{14}$ [f] |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.5 | 46 060 | 2 966 | 93.6 | 15.7 | 15.8 | 0.003 18 | 0.003 17 | 0.094 | 38.3 | 0.98 |
| 1.0 | 67 422 | 24 306 | 64.0 | 21.4 | 22.7 | 0.004 67 | 0.004 39 | 0.128 | 52.2 | 1.33 |
| 5.0 | 484 812 | 377 125 | 22.2 | 37.2 | 35.3 | 0.013 4 | 0.014 1 | 0.222 | 90.7 | 2.31 |
| 10.0 | 449 676 | 405 406 | 11.0 | 37.0 | 37.9 | 0.027 0 | 0.026 4 | 0.221 | 90.2 | 2.29 |
| 1.0[g] | 67 422 | 28 985 | 57.0 | 19.9 | | | | 0.114 | 46.6 | 1.18 |

[a] Particle size: 75–105 μm. [b] $100(A - B)/A$. [c] By eq 1. [d] By eq 2. [e] $100X/X_0$, where $X_0$ = extrapolated maximum adsorption (41.0 μg/g; Figure 1c). [f] Based on average surface area of 0.058 m²/g; see text. [g] Experiment conducted at 35 °C.

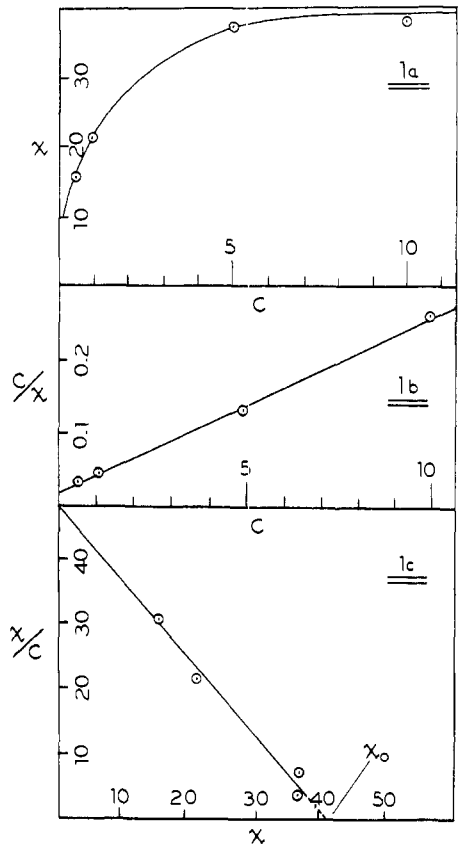



Figure 1. Langmuir adsorption isotherm plots for (*S*)-alanine isopropyl ester hydrochloride in chloroform (units in Table IV).

also in reasonable agreement with the surface area value, 0.11 m²/g, independently measured by the B.E.T.[17] method using nitrogen as the adsorbed gas. Using the overall average surface area of 0.058 m²/g for our 90 μm (average diameter) quartz particles, one can calculate that the area coverage of the quartz by (*S*)-**2** molecules varies in our experiments between ca. 1.0 and 2.3 × $10^{14}$ molecules/cm², as seen in the last column of Table IV.

Finally, we have repeated one of our 25 °C adsorption experiments ($10^{-4}$ M) in Table IV at 35 °C. As expected, the percent adsorption and the surface coverage are curtailed at the higher temperature. While sufficient data are not on hand for an accurate calculation, it is possible to make a rough estimate from the temperature effect in question that the differential heat of adsorption of (*S*)-**2** by quartz is in the neighborhood of 2.2 kcal/mol.

**Asymmetric Adsorption of (*R,S*)-Alanine Isopropyl Ester Hydrochloride.** Our final effort in this study was an attempt to confirm our original observations of the asymmetric adsorption of alanine hydrochloride (**1**) by *d*- and *l*-quartz[11,12] using a different amino acid and/or derivative, a different solvent system, and a different method of measuring asymmetric adsorption, namely, GC. The efficacy of the latter technique has been abundantly demonstrated in other studies where the very precise (0.1–0.2%) determination of the enantiomeric composition of mixtures of minute quantities of (*R*)- and (*S*)-amino acids has been required.[18,19] On the basis of conclusions from the experiments described above, we selected for this study to investigate the asymmetric adsorption of (*R,S*)-**2** by *d*- and *l*-quartz from its $10^{-4}$ M solution in chloroform at room temperature.

Preliminary experiments involving column chromatography were conducted, wherein we percolated under pressure chloroform solutions of labeled (*R,S*)-**2** through chromatographic columns of *d*- or *l*-quartz powder and then estimated gross adsorption by counting aliquots of the eluate for their radioactive solute. It was quickly apparent that reproducible results could not be obtained, presumably due to our inability to maintain consistently anhydrous conditions in loading the columns and to control channeling during elution. Accordingly batch process experiments were again employed, wherein such difficulties could be obviated.

Our typical procedure involved stirring an aliquot of $10^{-4}$ M chloroform solution of $^3$H-labeled (*R,S*)-**2** with *d*- or *l*-quartz powder, then determining the gross adsorption by radioactivity counting of the stock and supernatant solutions in the usual way. The supernatant was evaporated and the residue was trifluoroacetylated. GC analysis of the resulting **4**[18,20] then provided a determination of the enantiomeric composition of the unadsorbed material in the original supernatant. The residual quartz was dried, stirred with additional chloroform, and allowed to settle. The "first desorption" supernatant was similarly counted for radioactivity and its solute was recovered, trifluoroacetylated, and analyzed by GC as before. A second similar desorption using chloroform, followed by two analogous desorptions with methanol, was then undertaken, ultimately removing essentially all of the labeled adsorbate originally bound to the quartz. The content of **2** in the original supernatant and each subsequent desorption supernatant (>97% recovery) for a typical experiment involving *l*-quartz are shown in Table V, Part A, along with the GC-determined enantiomer content of each supernatant in question. Table V, Part B, reduces the analytical data of Part A to the percent recovery of each enantiomeric component for each supernatant examined. Table VI summarizes the results for the most pertinent fractions of several similar experiments involving both *d*- and *l*-quartz.

The first column of Table V, Part A, indicates that the *l*-quartz preferentially adsorbed (*R*)-**2** from the racemate; that is, the initial supernatant was richer in the (*S*) enantiomer.

**Table V.** Asymmetric Adsorption/Desorption of (*R,S*)-Alanine-$^3$H Isopropyl Ester Hydrochloride by *l*-Quartz

| | Supernatant from | | | | | |
|---|---|---|---|---|---|---|
| | Initial equil | Desorption with $CHCl_3$ | | Desorption with $CH_3OH$ | | Total, % |
| | | 1st | 2nd | 1st | 2nd | |
| (A) Total Adsorption, Enantiomeric Composition, Asymmetric Adsorption | | | | | | |
| % of total in supernatant[a] | 26.61 | 14.29 | 9.79 | 36.11 | 10.84 | 97.64 |
| % (*R*)[b] | 49.25 ± 0.25 | 45.57 ± 0.15 | 43.79 ± 0.15 | 51.17 ± 0.10 | 52.37 ± 0.28 | |
| % (*S*)[b] | 50.75 ± 0.25 | 54.43 ± 0.15 | 56.21 ± 0.15 | 48.83 ± 0.10 | 47.63 ± 0.28 | |
| % asymmetric adsorption[c] | −1.5 ± 0.35 | −8.86 ± 0.21 | −12.42 ± 0.21 | 2.34 ± 0.14 | 4.74 ± 0.40 | |
| (B) Percent of Total Recovered Material in Each Supernatant | | | | | | |
| (*R*) | 13.11 | 6.51 | 4.29 | 18.48 | 5.68 | 48.07 |
| (*S*) | 13.50 | 7.78 | 5.50 | 17.63 | 5.16 | 49.57 |
| Total % | 26.61 | 14.29 | 9.79 | 36.11 | 10.84 | 97.64 |

[a] Based on triplicate radioactivity counting of stock and supernatants. [b] Based on triplicate GC analyses corrected to triplicate analyses on (*R,S*) standard. [c] % (*R*) − % (*S*).

**Table VI.** Summary of Asymmetric Adsorption Experiments with (*R,S*)-Alanine-$^3$H Isopropyl Ester Hydrochloride

| Quartz | Supernatant from[a] | % of total in supernatant[b] | Enantiomeric composition[c] | | | Asymmetric adsorption, %[d] |
|---|---|---|---|---|---|---|
| | | | % (*R*) | % (*S*) | (±) | |
| *l*-[e] | A | 26.6 | 49.25 | 50.75 | 0.25 | −1.50 |
| | B | 14.3 | 45.57 | 54.43 | 0.15 | −8.86 |
| | C | 36.1 | 51.17 | 48.83 | 0.10 | +2.34 |
| *d*-[f] | A | 21.2 | 50.21 | 49.79 | 0.53 | +0.42 |
| | B | 13.9 | 54.09 | 46.91 | 0.72 | +8.18 |
| | C | 39.3 | 47.75 | 52.25 | 0.07 | −4.50 |
| *l*-[e] | A | 26.8 | [g] | | | |
| | B | 14.6 | 47.36 | 52.64 | 0.54 | −5.28 |
| | C | 20.5 | 54.31 | 45.69 | 0.86 | +8.62 |

[a] A, initial equilibration; B, first desorption with $CHCl_3$; C, first desorption with $CH_3OH$. [b] Based on triplicate radioactivity counting of stock and supernatants. [c] Based on triplicate GC analyses corrected to triplicate analyses for (*R,S*) standard. [d] % (*R*) − % (*S*). [e] 20 g; 105–125 μm particle size. [f] 20 g; 63–88 μm particle size. [g] Sample lost.

Similarly, when the residual quartz was successively rinsed with chloroform to remove adsorbed material, the (*S*) isomer desorbed preferentially. Thus, as seen in Table V, Part B, the initial supernatant and the two chloroform desorption supernatants contained 54.0% (i.e., 26.78/49.57) of the total (*S*) isomer recovered but only 49.7% of the more strongly bound (*R*) isomer. Subsequent elution of the remaining adsorbate by means of the final methanol rinses, conversely, afforded desorbed material richer in the (*R*) enantiomer, confirming the original conclusion that *l*-quartz preferentially adsorbs (*R*)-**2**. Part B also shows that the overall recovery of each enantiomer among all of the supernatants examined was nearly quantitative, 96.1% for the (*R*) and 99.1% for the (*S*) isomer.

The same conclusions arise from the two confirmatory experiments summarized (along with some of the data of Table V) in Table VI. Here we see that the chloroform supernatants are uniformly richer in (*S*) enantiomer and the methanol supernatant richer in the (*R*) enantiomer when *l*-quartz is used, while exactly the reverse is true when *d*-quartz is used. Finally, the "asymmetric adsorption" is seen to vary between −12.4 and +8.6% among the various supernatants examined in these experiments, depending upon the handedness of the quartz employed.

The implications of such successful asymmetric adsorption exeriments involving *d*- and *l*-quartz with respect to the earlier literature, as well as to the subjects of chemical evolution and the prebiotic origin of optical activity in nature, have been discussed in a previous communication.[11]

## Experimental Section

***d*- and *l*-Quartz Powder.** The quartz samples used in the present experiments were prepared exactly as described previously.[11,12]

**The Role of Amino and Carboxyl Groups in Adsorption.** The following three experiments were undertaken. For the first experiment 20 μl of a 5.4 × $10^{-5}$ M aqueous solution of (*S*)-alanine-3-$^3$H hydrochloride (30–50 Ci/mmol; No. NET-348; New England Nuclear, Boston, Mass.) was added to 1.5 ml of $10^{-2}$ M (*R,S*)-alanine in 1 N hydrochloric acid. The solution was evaporated to dryness under vacuum at 70 °C and the residual $^3$H-labeled (*R,S*)-**1** was treated with a large excess of 2-propanol saturated with hydrogen chloride (2-propanol:alanine ca. 100:1). The solution was heated under reflux for 45 min, whereupon the alcohol was evaporated and the residue was dissolved in 15 ml of anhydrous DCM to give a $10^{-3}$ M stock solution of $^3$H-labeled (*R,S*)-**2**. (The DCM used in these experiments was dried over 5 Å Molecular Sieve and stored under septum. It contained less than 0.5 ppm water, as shown by adding 10 μl $^3H_2O$ to 20 ml of solvent, then counting for radioactivity before and after drying; 99.9% of the label was removed during drying.)

For the second experiment, a dried sample of $^3$H-labeled (*R,S*)-**1** was prepared as described above and then dissolved in 5 ml of DCM and treated with 0.5 ml of trifluoroacetic anhydride. After 2 h the solvents were stripped and the residue was dissolved in 15 ml of DCM to give a $10^{-3}$ M stock solution of $^3$H-labeled (*R,S*)-**3**. For the third experiment, labeled (*R,S*)-**2** was prepared as above and was then trifluoroacetylated in the manner described for the preparation of the (*R,S*)-**3**. The product was again dissolved in 15 ml of DCM to give a $10^{-3}$ M stock solution of $^3$H-labeled (*R,S*)-**4**.

A 300-μl aliquot of each stock solution was counted for radioactivity during a 2-min period as previously described.[11] Ten milliliters of each

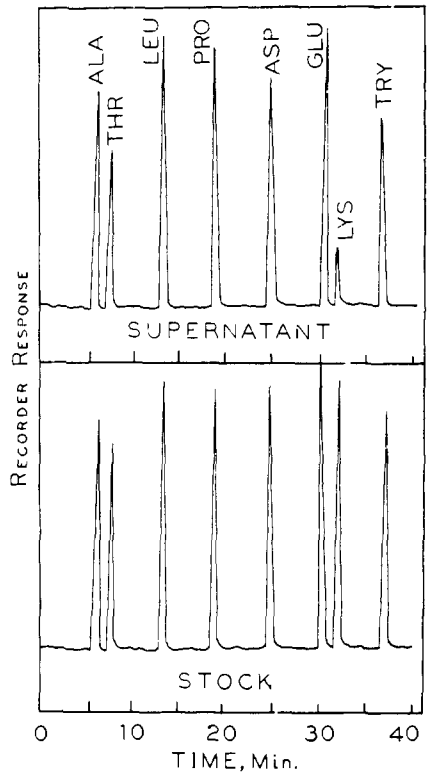



Figure 2. Gas chromatographic analyses of *N*-TFA-amino acid *n*-butyl esters from set III, supernatant and stock solutions.

stock solution in turn was then introduced through a septum onto a 5-g sample of dried *l*-quartz (particle size <45 μm) contained in a 25-ml flask attached to a vacuum line.[11] Each mixture was then stirred magnetically for 60 min and allowed to settle for 3 h, whereupon a 300-μl aliquot of each supernatant was removed and similarly counted for radioactivity for a 2-min period. The results of the three experiments performed are summarized in Table I.

**Comparative Adsorption of Amino Acid *n*-Butyl Ester Hydrochlorides.** Ten different amino acids (representing neutral, acidic, and basic types) were divided into three sets of mixtures designed for ease of gas chromatographic analysis, depending on the known gas chromatographic elution patterns of their *N*-TFA *n*-butyl ester derivatives on a Dexsil-400 capillary column.[21] The three sets contained approximately equimolar mixtures of the components shown in Table II. Each mixture was dissolved in a small quantity of 1 N hydrochloric acid, then evaporated to dryness and treated with an excess of *n*-butyl alcohol saturated with hydrogen chloride. After 1 h reflux the volatiles were evaporated and the residues were each dissolved in 30 ml of anhydrous DCM to provide the stock solution for each set. The concentration of each *n*-butyl ester in each stock solution was ca. $2 \times 10^{-4}$ M. Fifteen milliliters of each stock solution was then stirred with 6 g of dried, powdered *d*-quartz (particle size <45 μm) for 1 h in the usual apparatus, then allowed to settle for 3 h. Three 200-μl aliquots of each supernatant were removed in turn and counted for radioactivity as before, and three 200-μl aliquots of each original stock solution were similarly counted. The count loss in each case provided a direct measure of the percent adsorption of the labeled amino ester in each set, which provided the internal standard in the GC analyses below. The amino esters in each stock solution and each corresponding supernatant were then converted to their *N*-TFA derivatives by addition of 0.5 ml of trifluoroacetic anhydride to each solution. After 2 h the volatiles were evaporated and the residues were dissolved in sufficient nitromethane to provide ca. $10^{-2}$ M solutions. These were analyzed by GC (each solution in triplicate with stock and supernatants run "back to back") using a 0.02 in. × 150 ft capillary column coated with the Dexsil-400 phase and installed in a Hewlett-Packard 5700A Gas Chromatograph. Peak area integration was accomplished with an Autolab 6300 Digital Electronic Integrator, while monitoring each peak with a Varian A25 20-speed recorder. Typical gas chromatograms for the stock solution and supernatant corresponding to set III are shown in Figure 2.

The "exact" percent adsorption for the *labeled* amino ester in each

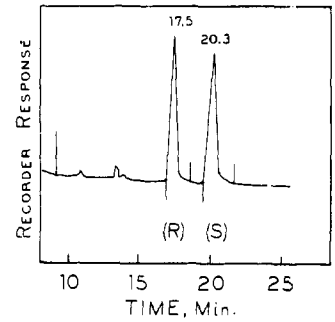



Figure 3. Typical gas chromatographic resolution of *n*-TFA-(*R,S*)-alanine isopropyl ester using 150 ft capillary column coated with *N*-lauroyl-L-valyl-*tert*-butylamide phase.

set was established by liquid scintillation counting of each stock and supernatant solution, as described above. Using such values as the internal standard for each set, it was then possible to calculate "corrected" GC peak areas for the *N*-TFA amino esters in the supernatants of each set. Dividing these corrected areas by the peak areas of the corresponding components in the stock solutions, then subtracting from 100%, gave a direct GC measurement of the percent adsorption of each amino ester in each set. Table II summarizes the data for the three sets of amino acid *n*-butyl ester mixtures studied.

**Adsorption Using Different Solvent Systems.** Solutions of $^3$H-labeled (*R,S*)-**2** ($10^{-4}$ M) were prepared in the pure or mixed solvents (dried as before) listed in Table III, and each stock solution was counted in triplicate for radioactivity. Two milliliters of each solution was then stirred with 1.5–2.0 g of *l*-quartz (105–125 μm particle size) for 1 h in the usual apparatus and was then allowed to settle, and each supernatant was similarly counted for radioactivity. The results are summarized in Table III.

**Adsorption as a Function of Concentration and Temperature.** Aliquots of chloroform solutions (10 ml) of $^3$H-labeled (*S*)-**2** varying in concentration from $5 \times 10^{-5}$ to $10^{-3}$ M were each equilibrated by stirring for 1 h with 5 g of dried *l*-quartz (particle size, 75–105 μm), and the radioactivity of each stock and supernatant solution was measured as before to estimate gross adsorption in each experiment. Other data of interest, derivable from the gross adsorption and concentration data, are summarized in Table IV.

The specific surface area of the quartz sample used in the above experiments was established independently to be 0.11 $m^2/g$, employing the previously described[11] B.E.T. method[17] and using nitrogen as the adsorbed gas.

**Asymmetric Adsorption of (*R,S*)-Alanine Isopropyl Ester Hydrochloride.** The following procedure was typical. A stock solution consisting of 32 ml of $10^{-4}$ M $^3$H-labeled (*R,S*)-**2** in anhydrous chloroform was prepared as described above. A 300-μl aliquot of this solution was counted in triplicate for radioactivity, whereupon 10 ml was removed and stripped of solvent. The residue was converted into its *N*-trifluoroacetyl derivative, which was dissolved in nitromethane to provide a (*R,S*)-**4** standard for subsequent GC analyses of enantiomeric composition. A 20-ml sample of the remaining stock solution was stirred for 1 h with 20 g of dried *l*-quartz powder (105–125 μm) under anhydrous conditions in the usual apparatus.[11] The mixture was allowed to settle for 3 h, whereupon 15 ml of the supernatant was decanted and three 300-μl aliquots were removed therefrom for triplicate radioactivity counting to estimate solute concentration and gross adsorption. The remainder of the decanted supernatant was stripped of solvent and the residue was trifluoroacetylated and dissolved in nitromethane as before for GC analysis of enantiomeric composition. The residual quartz (containing 25% of the supernatant) was dried under aspirator vacuum, then was washed by stirring with 20 ml of fresh chloroform and allowed to settle. Fifteen milliliters of the second supernatant was then removed and handled exactly as was the original supernatant. The process was again duplicated with 20 ml of fresh chloroform, then was finally repeated twice using 20-ml portions of methanol. Radioactivity counting of the methanol rinses was accomplished after evaporating the 300-μl aliquots of methanol solution and redissolving the residue in 300 μl of chloroform.

GC analyses for the enantiomeric composition of the (*R*)- and (*S*)-**4** mixture from each of the supernants above were conducted each in triplicate using a 0.02 in. × 150 ft capillary column coated with *N*-

lauroyl-(*S*)-valyl-*tert*-butylamide optically active stationary phase[20] (30 mg; from a 5% solution in DCM) (Miles Laboratories, Inc., Kankakee, Ill.) and installed in the Hewlett-Packard 5700A gas chromatograph. This was operated isothermally at 100 °C with a helium flow rate of 5 ml/min, and peak tracing and integration were accomplished with a Hewlett-Packard 3800A digital electronic integrator-recorder. As seen in Figure 3, these conditions permitted baseline resolution of the (*R*) and (*S*) enantiomers within ca. 22 min, with a peak separation of some 2.8 min. The results of this experiment are presented in Table V, while Table VI includes similar results from two replicate experiments.

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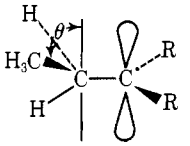
# Contact Chemical Shifts for the Carbon Atoms of Nickel Complexes of the 4-Alkylanilines. The Factors Governing the EPR Hyperfine Constants of Carbon Atoms[1]

**Leon M. Stock* and Michael R. Wasielewski[2]**

*Contribution from the Department of Chemistry, University of Chicago, Chicago, Illinois 60637.* *Received June 4, 1976*

**Abstract:** The proton and carbon contact chemical shifts of the nickel acetylacetonate complexes of 20 aniline derivatives have been measured. The signs and magnitudes of the contact shifts for the meta and para carbon atoms of these anilines are consistent with the dominant $\pi$ delocalization of spin density. The contact chemical shifts for the $\alpha$ carbon atoms of the substituents in 4-alkylaniline derivatives depend on the hybridization of the bonding orbital of the $\alpha$-carbon atom and on the degree of substitution of the $\alpha$ atom. The contact chemical shifts for the $\beta$-carbon atoms in 4-alkylaniline derivatives exhibit an angular dependence. The results for molecules in which the dihedral anle is defined by structural constraints are well described by $a_\beta^C = \rho_C^\pi(-1.1 + 23\langle\cos^2\theta\rangle)$. In general, the contact chemical shifts both for the $\beta$-hydrogen and the $\beta$-carbon atoms of 4-alkyl groups conform to relationships based on $\langle\cos^2\theta\rangle$. The shifts for the 4-cyclopropylanilines deviate from this relationship. These data indicate that more spin density is delocalized to cyclopropyl groups in the bisected conformation than in the perpendicular conformation. The concept of carbon–carbon hyperconjugation provides a basis for the interpretation of the results.

Interest in the relative importance of carbon–carbon compared to carbon–hydrogen hyperconjugation prompted the initial studies of the electron paramagnetic resonance hyperfine constants, $a_\beta^C$, for $\beta$-carbon atoms in radicals.[3-5] The results




established that spin delocalization to $\beta$-carbon atoms is just as important as spin delocalization to $\beta$-hydrogen atoms.[3] Progress in the definition of the factors governing spin delocalization to $\beta$ carbon atoms has been impeded by the difficulties inherent in the spectroscopic study of $^{13}C$ in natural abundance and in the synthesis of enriched compounds. Consequently, there are few reports concerning these hyperfine interactions.[6-10] The work has, for the most part, focused on the definition of $B_2^C$ in eq 1, where $\rho_C^\pi$ is the spin density in the adjacent p orbital, $\theta$ is the dihedral angle, and $B_0^C$ and $B_2^C$ are empirical constants.

$$a_\beta^C = \rho_C^\pi(B_0^C + B_2^C\langle\cos^2\theta_C\rangle) \quad (1)$$

Experimental estimates of the angle-dependent term, $B_2^C$, which reflects the extent of spin delocalization range from about 15 to 25 G.[5-8] In the absence of experimental information most workers have assumed that the angle-independent term, $B_0^C$, is negligible.[6-8] For comparison, the theoretical results for the 1-propyl radical suggest that $a_\beta^C$ should be linearly dependent on $\langle\cos^2\theta_C\rangle$ with $B_0^C = 1.1$ G and $B_2^C = 13.8$ G.[11]

New interest in the concept of carbon–carbon hyperconjugation as an important factor governing the reactivity of organic molecules indicated that a thorough investigation of the hyperfine interactions of the $\beta$ carbon atoms would be useful to gauge the impact of steric effects, hybridization changes,