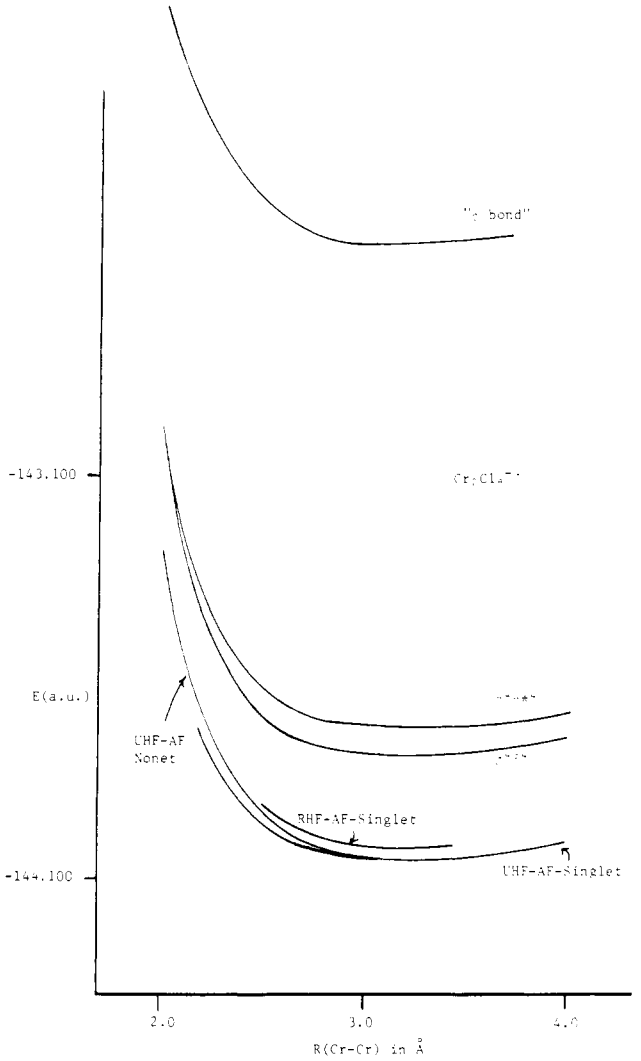


**Figure 5.** Summary of results for calculations on $Cr^2Cl_8^{4-}$ see text.

while the latter is well characterized.[10]

Although the minimum of our potential is between 2.5 and 3.5 Å for any reasonable choice of four point charges or four Li atoms and these potential energy curves are relatively flat, experimental bond lengths are shorter and are reported between 1.85 and 2.6 Å. The reason for this discrepancy is not clear. From the experimental side these flat potentials may be greatly influenced by crystal forces: we have calculated for only a single molecule. From the theoretical side, basis-set deficiencies or correlation effects may be operative. We find, however, that no reasonable change in INDO parameters changes these geometry predictions significantly, and such changes usually mimic basis-set effects. Preliminary studies with much larger CI's do suggest a shortening of the bond, but only by about 0.3 Å.

Although the importance of the antiferromagnetic state might be expected to decrease with decreasing Cr–Cr bond distance (and increasing 3d–3d orbital interaction), this decrease is not significant for bond lengths as short as 2.0 Å. This is obvious from an examination of the spin density matrix or the expectation value of $S^2$ ($\langle S^2 \rangle$ = 3.736 at 2.0 Å). For completeness we also mention that small basis sets generally favor spin polarization in a UHF treatment that may disappear as the size of the basis set is increased. The energy lowering accompanying spin polarization in cases in which there are basis set deficiencies, however, is never as large as we find for these Cr–Cr complexes. The antiferromagnetic state is clearly lowest in energy in the entire range of Cr–Cr bond lengths studied (Figure 5).

(10) A. P. Sattelberger and J. P. Fackler, *J. Am. Chem. Soc.*, **99**, 1258 (1977).

No extrapolation of these results can yet be made for the molybdenum complexes since the d orbitals of Mo are considerably larger than those on Cr. The larger d orbitals would result in larger d–d overlap and suggests the possibility of stronger covalent coupling between Mo centers. The much more uniform distribution of the bond lengths in the Mo–Mo complexes may in fact be an indication that $\delta$ bonding is important for these compounds.[9,11]

Recent MC SCF (multiconfiguration self-consistent field) calculations by Goodgame and Goddard on the "sextuple bond" of $Cr_2$[12] are in essential agreement with our findings on the "$\delta$-bonded" $Cr_2X_8^{4-}$ complexes examined here in that they emphasize the importance of antiferromagnetic coupling in Cr–Cr bonding. It is interesting to note that their calculated Cr–Cr bond length is between 3.06 and 3.25 Å depending on the method utilized, much longer than the reported length of 1.7 Å. The experimental evidence for this short bond length, however, is not strong.[12,13]

**Acknowledgment.** This work was sponsored in part through the Natural Sciences and Engineering Research Council of Canada and CNPq Brazil.

**Registry No.** $Cr_2Cl_8^{4-}$, 63448-50-0.

(11) The reference list above is in no sense complete, as literally hundreds of valuable contributions on the subject of multiply bonded binuclear systems have appeared in the last few years.

(12) M. M. Goodgame and W. A. Goddard, III, *J. Phys. Chem.*, **85** 215 (1981).

(13) Y. M. Efremo, A. N. Samoilova, and L. V. Gurvich, *Opt. Specktrosk.*, **36**, 654 (1974).

## Biosynthesis of Sulfur Compounds. Investigations of the Biosynthesis of Asparagusic Acid

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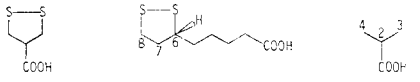
Asparagusic acid (**1**) is a naturally occurring 1,2-dithiolane that




**1** **2** **3**

has been isolated from both the roots and edible portions of asparagus plants (*Asparagus officinalis* L.).[1-3] The substance is a plant-growth inhibitor, exerting activity comparable to abscisic acid,[1,2] and it also possesses potent nematicidal activity.[3] Previous investigations of the biosynthesis of naturally occurring 1,2-dithiolanes appear to have been confined to the enzyme cofactor $\alpha$-(+)-lipoic acid (**2**).[4-7] We now report the results of experiments that show that the 1,2-dithiolane ring system of asparagusic acid is biosynthesized by a different mechanism than is involved in the biosynthesis of lipoic acid.

Our initial investigation of the biosynthesis of asparagusic acid was based upon a presumed analogy with lipoic acid biosynthesis. Since octanoic acid is the precursor of lipoic acid,[4] it was hy-

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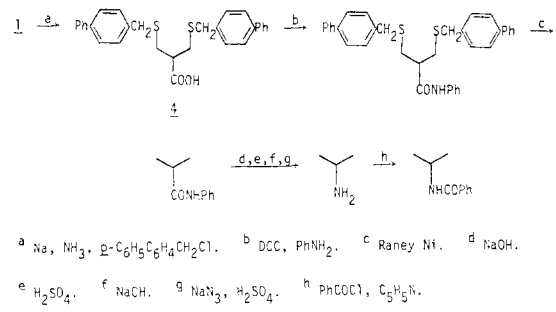
**Table I.** Incorporation of Radiolabeled Precursors into Asparagusic Acid

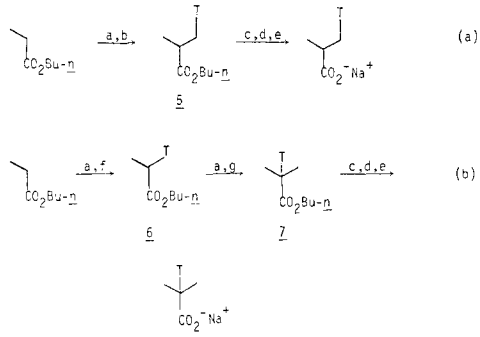
| expt no. | precursor ($^3H/^{14}C$) | % incorpn or $^3H/^{14}C$ | labeling pattern or % $^3H$ retention |
|---|---|---|---|
| 1 | sodium [1-$^{14}$C] isobutyrate | 1.2% | no radioactivity in C-2, C-3, or C-4 |
| 2 | sodium [3,4-$^3$H, 1-$^{14}$C] isobutyrate (5.72) | $^3H/^{14}C = 5.53$ | 96.7% $^3H$ retention |
| 3 | sodium [2-$^3$H, 1-$^{14}$C] isobutyrate (6.36) | $^3H/^{14}C = 0.16$ | 2.5% $^3H$ retention |
| 4 | sodium [1-$^{14}$C] methacrylate | 0.38% | no radioactivity in C-2, C-3, or C-4 |

**Scheme I**




[a] Na, $NH_3$, p-$C_6H_5C_6H_4CH_2Cl$. [b] DCC, $PhNH_2$. [c] Raney Ni. [d] NaOH. [e] $H_2SO_4$. [f] NaOH. [g] $NaN_3$, $H_2SO_4$. [h] PhCOCl, $C_5H_5N$.

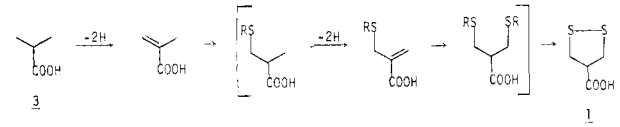
**Scheme II**




[a] LDA, THF-HMPA. [b] $^3H$–$CH_3I$. [c] NaOH. [d] $H_2SO_4$. [e] NaOH. [f] $^3H$–$CF_3CO_2H$. [g] $CH_3I$.

pothesized that isobutyric acid (**3**) would prove to be the precursor of asparagusic acid. Accordingly, commerically available sodium [1-$^{14}$C]isobutyrate was administered to young *Asparagus officinalis* plants by the cotton-wick method, and asparagusic acid was isolated after 4 days by dilution with synthetic asparagusate.[8] The recovered asparagusic acid was derivatized to yield the bis(*p*-phenylbenzyl) thioether **4** (Scheme I). Purification of **4** by chromatography and repeated crystallization gave the incorporation figure shown in Table I (experiment 1). Degradation of the derivative **4** was carried out according to Scheme I to prove that isobutyric acid had been specifically incorporated into asparagusic acid (Table I, experiment 1). Experiments with tritiated forms of isobutyric acid were then carried out to obtain clues to the mechanism of sulfur introduction. Sodium [3,4-$^3$H]isobutyrate was synthesized as outlined in Scheme IIa. Treatment of *n*-butyl propionate[9] with LDA in THF–HMPA[10] followed by alkylation with [$^3$H]methyl iodide yielded *n*-butyl[3,4-$^3$H]isobutyrate (**5**). Base-catalyzed hydrolysis of **5** followed by acidification, steam distillation, and titration with alkali yielded the desired sodium [3,4-$^3$H]isobutyrate (16% overall radiochemical yield). Sodium [2-$^3$H]isobutyrate was synthesized in a similar fashion (Scheme IIb); the anion formed from *n*-butyl propionate and LDA was quenched with [$^3$H]trifluoroacetic acid to generate *n*-butyl[2-$^3$H]propionate (**6**); deprotonation of **6** with LDA was then followed by alkylation with unlabeled methyl iodide to produce *n*-butyl-[2-$^3$H]isobutyrate (**7**). The labeled ester **7** was finally converted into sodium[2-$^3$H]isobutyrate by hydrolysis, steam distillation,

**Scheme III**




and titration (4.4% overall radiochemical yield). Each of the samples of specifically tritiated sodium isobutyrate was mixed with sodium[1-$^{14}$C]isobutyrate, and the tritium:carbon-14 ratios were determined by direct methods and by derivatization of a portion of each mixture as the corresponding anilide. The anilides derived from each mixture were recrystallized to constant activity and constant ratio. In each case, the tritium:carbon-14 ratios obtained directly were identical within experimental error with the ratios obtained by recrystallization of the anilides. The two samples of doubly labeled sodium isobutyrate were then administered to *Asparagus*, and asparagusic acid was isolated and derivatized as described above. The results of these experiments are shown in Table I (experiments 2 and 3). The incorporation of [3,4-$^3$H]-isobutyrate into aspargusic acid proceeded without tritium loss, within experimental error. The high degree of tritium retention observed in this experiment is presumably the consequence of a substantial tritium isotope effect associated with the removal of a hydrogen atom from the methyl groups of isobutyrate. A similar degree of tritium retention was observed to result from the incorporation of [8-$^3$H]octanoic acid into lipoic acid.[4] On the other hand, the conversion of sodium [2-$^3$H]isobutyrate into asparagusate resulted in the loss of virtually all of the tritium label. This result stands in complete contrast to the behavior observed during lipoic acid biosynthesis. In the case of the latter 1,2-dithiolane, no tritium is lost from carbon atoms adjacent to the sites of sulfur introduction.[4] A plausible explanation for the loss of tritium from sodium [2-$^3$H]isobutyrate would involve dehydrogenation to methacrylic acid, a process that is known to occur in animals and in microorganisms.[11,12] Therefore, sodium [1-$^{14}$C]methacrylate was synthesized from isopropenylmagnesium bromide and [$^{14}$C]carbon dioxide and administered to *Asparagus* plants. After 4 days, radioactive asparagusic acid was obtained (Table I, experiment 4). Degradation of the asparagusic acid in the usual way (Scheme I) proved that the incorporation was specific.

The results of the aforementioned experiments suggest the biosynthetic pathway for asparagusic acid outlined in Scheme III. Dehydrogenation of isobutyric acid (or isobutyrylcoenzyme A) to methacrylic acid could be followed by the Michael-type addition of an unknown sulfur nucleophile. A second dehydrogenation step would then lead to an intermediate that could undergo addition of a second mole of the sulfur nucleophile to ultimately yield asparagusic acid. Investigations are in progress to determine the nature of the sulfur nucleophile and of the intermediates on the pathway between methacrylic acid and asparagusic acid.

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**Registry No. 1**, 2224-02-4; **3**, 79-31-2; sodium [1-$^{14}$C]isobutyrate, 6917-21-1; sodium [3,4-$^3$H]isobutyrate, 80631-54-5; sodium [2-$^3$H]isobutyrate, 80631-55-6; sodium [1-$^{14}$C]methacrylate, 80631-56-7.

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