$$(4)$$

$$(5)$$

reported overall reaction stoichiometry, but also for several peculiar aspects of O_2 uptake and generation.[13]

Other reported cases of apparent oxidation by $\cdot O_2^-$ include hydrazines,[9,16,17] thiols,[7b,18,22] and certain alcohols.[7b,22] For each of these, proton transfer to $\cdot O_2^-$ appears to be a necessary first step (dialkyl sulfides and ethers are inert, and alkoxides are inert). In fact, Misra and Fridovich[24] have established that protons or metal cations are necessary to catalyze the oxidation of hydrazine by superoxide.

Effective Basicity. A second property of superoxide solutions is their capacity to effect proton removal from substrates.[24] For example, eq 6, which represents the acid-catalyzed disproportionation of superoxide, has a K value of 2.5×10^8 when the substrate is H_2O.[20] Obviously, even much weaker acids than water will produce an exothermic reaction here.

$$2 \cdot O_2^- + HB \overset{K}{\rightleftharpoons} O_2 + HO_2^- + B^- \qquad (6)$$

The overall reactions represented by eq 6 may not be rapid with weak acids (HB), but neither are many of the reported substrate reactions with $\cdot O_2^-$. Indeed, recent data in our laboratory establish that a number of weakly acidic organic compounds are deprotonated efficiently in the presence of superoxide, especially when the organics are solvents or in high concentrations.[22]

The cleavage of esters by superoxide ion recently has been described as a nucleophilic attack.[7b] However, in the presence of water or other proton sources superoxide ion could lead to the formation of strong Brønsted bases via reaction 6 to give ester hydrolysis without direct attack by $\cdot O_2^-$.

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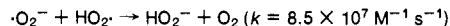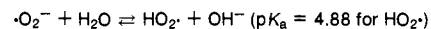(20) The combination of the reduction half-reaction for $\cdot O_2^-$ in water[21]

$\cdot O_2^- + H_2O + e^- \rightarrow HO_2^- + OH^-$ $E^{\circ\prime} = -0.08$ V
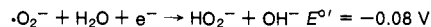
with eq 1 (the redox potential of $O_2/\cdot O_2^-$ in water, $E^{\circ\prime}$, is -0.58 V)[21] yields the net expression

$2 \cdot O_2^- + H_2O \rightleftharpoons O_2 + HO_2^- + OH^-$ $K = 2.5 \times 10^8$
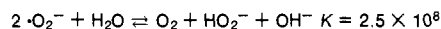
This indicates that superoxide ion solutions can promote proton transfer from substrates to an extent equivalent to that for the conjugate base of an acid with an approximate pK_a value of 23 (assuming a pK_a of 15 for water). This equilibrium expression is the result of the reactions

$\cdot O_2^- + H_2O \rightleftharpoons HO_2\cdot + OH^-$ ($pK_a = 4.88$ for $HO_2\cdot$)
$\cdot O_2^- + HO_2\cdot \rightarrow HO_2^- + O_2$ ($k = 8.5 \times 10^7$ M^{-1} s^{-1})

which are well established.[19] Note that the last reaction is rapid and highly exothermic. Because of this, the lifetime of $HO_2\cdot$ should be sufficiently short to preclude it as a major reactant with most substrates in solution.
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Structure of Shikodonin, a Unique Anti-Tumor Spirosecokaurene Diterpenoid

Sir:

Recently we have reported the structure of a new *ent*-kaurene diterpenoid, shikokianidin,[1] which was isolated in addition to shikokianin and oridonin from the dry leaves of the ether extracts of *Isodon shikokianus* (Labiatae).[2] Further examination of the same plant has now resulted in the isolation of another new diterpenoid, shikodonin, in 0.00005% yield. This minor bitter principle could only be separated via chromatography on silica gel followed by HPLC using μ-Bondapak-C_{18}, 4-mm i.d. $\times$ 30 cm, methanol–water (42.5:57.5 v/v).

Shikodonin possesses significant in vitro cytotoxicity (KB) and in vivo antitumor activity against Ehrlich ascites carcinoma inoculated into mice.[3,4] It also exhibits insect growth inhibitory activity specifically against Lepidopteran larvae. For example, shikodonin much more strongly inhibits the respiratory reactions of mitochondria from *Bombyx mori* than from mammalian tissue (rat liver).[5]

We have now established the unique spirosecokaurene structure **1** for shikodonin, with the following physical properties: $C_{20}H_{26}O_6$ (chemical ionization–mass spectroscopy in isobutane and elemental analysis); mp 206–209 °C; UV (EtOH) 233.5 nm (ϵ 8880); IR (Nujol) 3550 (hydroxyl), 1730 (six-membered lactone), 1700 and 1640 cm^{-1} (five-membered ring ketone conjugated with an exocyclic methylene). The ^{13}C NMR data of shikodonin showed the presence of one methyl, seven methylenes, five methines, and three tetrasubstituted carbons, together with two olefinic and two carbonyl carbon atoms.[6] The pertinent ^1H NMR and ^{13}C NMR data are shown in Chart I.

Addition of the ^1H NMR shift reagent Eu(dpm)$_3$ caused a clear downfield shift of the 9β-H (d, 6 H). The magnitude of the coupling constants, $J_{9\beta,11\beta} = 6$, $J_{11\beta,12\alpha} = 15$, and
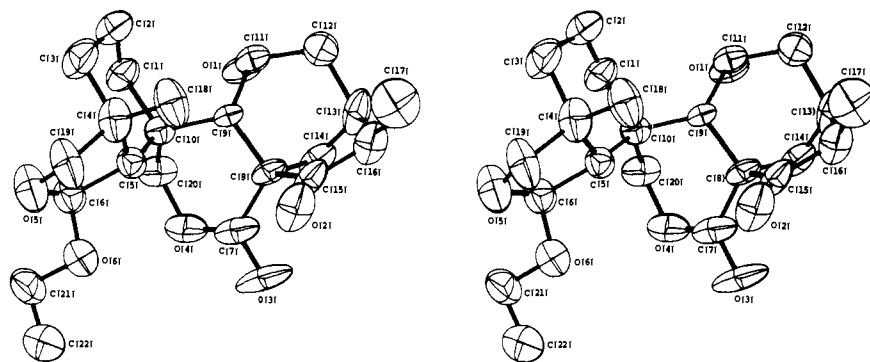
Figure 1. A stereodiagram of *O*-ethyldihydroshikodonin (**4**). No hydrogen atoms are shown.

Table I. Crystal Data for **2** and **4**

	2	4
Molecular formula	$C_{21}H_{28}O_6$	$C_{22}H_{32}O_6$
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1$	$P2_1$
a, Å	7.538 (3)	7.651 (2)
b, Å	9.244 (4)	9.649 (3)
c, Å	13.795 (6)	14.340 (5)
β, degree	103.83 (3)	101.07 (2)
Z	2	2
Volume, Å^3	933.4	1038.9
No. of independent reflections	1242	1391

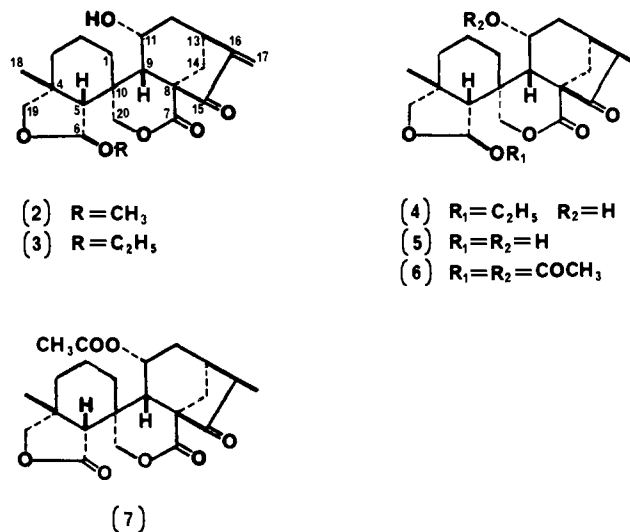**Chart I.** ^{1}H and ^{13}C NMR of Shikodonin Obtained in C_5D_5N
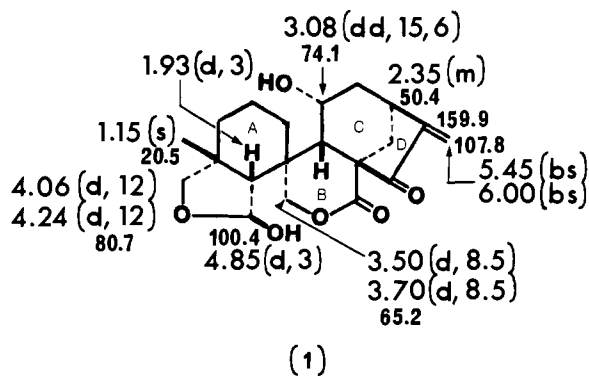
$J_{11\beta,12\beta} = 0$ Hz, made it obvious that the hydroxyl group was axial (α).

It should be noted that crystallization of **1** from methanol yielded mainly *O*-methylshikodonin (**2**), but from ethanol it gave *O*-ethylshikodonin (**3**). Similarly, catalytic hydrogenation of **1** in ethanol produced *O*-ethyldihydroshikodonin (**4**),[7] while in dioxane it afforded dihydroshikodonin (**5**). Acetylation of **5** with acetic anhydride–pyridine gave diacetyldihydroshikodonin (**6**) with no OH absorption. Hydrolysis of **6** with oxalic acid in dioxane–water (1:1 v/v), followed by oxidation with Jones reagent, yielded monoacetyldihydrolactone (**7**). The presence of an IR band at 1780 cm^{-1} confirmed that **7** had a five-membered-ring lactone.

The detailed structure and stereochemistry of shikodonin were established unambiguously by x-ray diffraction methods performed on *O*-methylshikodonin (**2**) and *O*-ethyldihydroshikodonin (**4**). The crystal data of **2** and **4** are summarized in Table I.

Intensities for both crystals were collected on a Philips four-circle diffractometer by the $\omega/2\theta$ scan technique with graphite-monochromated Mo Kα radiation for maximum 2θ angle of 55°. Both structures were solved by multisolution tangent refinement methods.[7] Block-diagonal least-squares refinements with anisotropic temperature factors for nonhy-

drogen atoms and isotropic hydrogens converged to standard crystallographic residuals of 0.059 for **2** and 0.052 for **4**.[8,9] Additional crystallographic details can be found in the supplemental material.

A stereoscopic view of the molecule **4** is shown in Figure 1 and ring designation is indicated in structure **1** (Chart I). The ring conformations of **4** are as follows: ring A is in a chair form and ring B is in a 1,3-diplanar form with the torsional angles of C(7)-C(8)-C(9)-C(10) = 1.7°, and C(20)-O(4)-C(7)-C(8) = 4.3°. Ring C is almost in a boat form since C(8), C(9), C(12), and C(13) are coplanar within 0.035 Å, and C(11) and C(14) deviate by 0.618 and 0.842 Å, respectively, in the same direction from this plane. The five-membered ring D has an envelope shape with C(14) serving as the flap, while the acetal ring is in a half-chair form. Compound **2** has the same ring conformation as molecule **4**, although some of the corresponding torsional angles are significantly different.

In view of the fact that over 45 *ent*-kaurene type compounds have been isolated from the *Isodon* genus, three from this particular species (*shikokianus*), with no kaurene compounds found, it seems reasonable to assume that the absolute stereochemistry of shikodonin is that of an *ent*-kaurene.

In addition to the unique spiro skeleton, shikodonin is of interest in that it is the first instance of an *ent*-kaurene type diterpenoid oxygenated at C-19 while all the other known kaurene type diterpenoids isolated from *Isodon* species are not oxygenated at C-19.

Supplementary Material Available: The crystallographic data for *O*-ethyldihydroshikodonin and *O*-methylshikodonin—fractional

coordinates, bond distances, bond angles, and observed and calculated structure factors (13 pages). Ordering information is given on any current masthead page.

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Copper Complex Acting as a Reversible Carbon Dioxide Carrier

Sir:

Use of CO_2 in synthetic organic reactions is important. Activation of CO_2 and its transfer to organic substrates by means of a transition metal complex acting as a reversible CO_2 carrier may be a promising approach. Among biological carboxylations, the biotin-dependent carboxylations are known to involve the intermediate formation of enzyme-bound carboxybiotin as a reversible carrier of activated CO_2.[1] Despite several recent reports on transition metal complexes having an ability for reversible CO_2 fixation,[2] examples effecting organic reactions of the CO_2 fixed in these complexes are few.[3] Here we report both a reversible decarboxylation of a copper(I) cyanoacetate–phosphine complex and a transcarboxylation by the complex to cyclohexanone.

All experiments described below were carried out under nitrogen atmosphere. Previously, we reported that copper(I) cyanoacetate underwent a quantitative and irreversible decarboxylation in dimethylformamide (DMF) at 50 °C to give isolable cyanomethylcopper(I).[4] Now it has been found that, in the presence of a PBu$_3{}^n$ ligand which dissolves copper(I) cyanoacetate in organic solvents, the decarboxylation of copper(I) cyanoacetate becomes reversible.

$$NCCH_2CO_2Cu \cdot (PBu_3{}^n)_x$$
$$\rightleftharpoons NCCH_2Cu \cdot (PBu_3)_x + CO_2 \quad (1)$$

The reversibility was supported by the following experimental results. (i) Cyanomethylcopper(I) absorbed CO_2 gas in the presence of 3 equiv of PBu$_3{}^n$ at 0 °C and methyl cyanoacetate was produced in a yield of 53% on treating the reaction mixture with methyl iodide at 0 °C to room tempera-
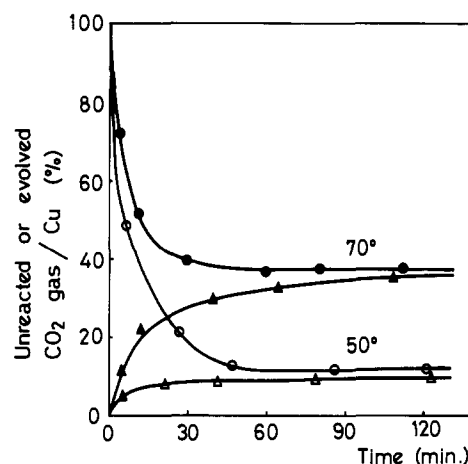

Figure 1. Reversibility of the carboxylation starting from 0.86 mmol of NCCH$_2$Cu (O) and 0.77 mmol of NCCH$_2$Cu (●) (ratio of PBu$_3{}^n$/Cu = 3), and the decarboxylation starting from 0.95 mmol of NCCH$_2$COOCu (▲) and 0.89 mmol of NCCH$_2$COOCu (△) (ratio of PBu$_3{}^n$/Cu = 3) in 5 mL of DMF: NCCH$_2$COOCu·(PBu$_3{}^n$)$_x$ $\rightleftharpoons$ NCCH$_2$Cu·(PBu$_3{}^n$)$_x$ + CO$_2$.
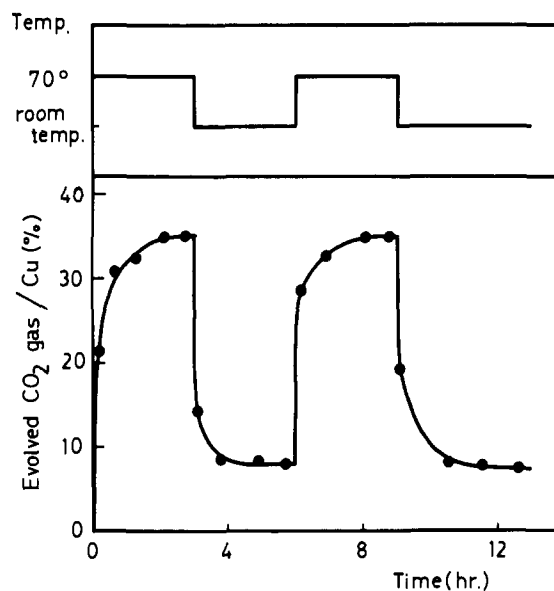
Figure 2. Cycle of CO$_2$ gas evolution–absorption by alternate heating and cooling of NCCH$_2$CO$_2$Cu·(PBu$_3{}^n$)$_x$: ratio of PBu$_3{}^n$/NCCH$_2$CO$_2$Cu (1.30 mmol) = 3; 5 mL of DMF.

ture. (ii) As shown in Figure 1, nearly the same state of equilibrium was attained from either direction of eq 1. (iii) A cycle of carboxylation–decarboxylation caused by changing the reaction temperature was repeatable (Figure 2).

As the molar ratios of PBu$_3{}^n$/NCCH$_2$CO$_2$Cu and PBu$_3{}^n$/NCCH$_2$Cu were increased from 1 to 2 and 3, the decarboxylation was depressed and the carboxylation was favored. However, an addition of 1 more equiv of PBu$_3{}^n$ beyond PBu$_3{}^n$/Cu = 3 did not cause any effect on the decarboxylation of PBu$_3{}^n$/NCCH$_2$CO$_2$Cu = 3 and the carboxylation of PBu$_3{}^n$/NCCH$_2$Cu = 3. Based on these findings, it might be assumed that three PBu$_3{}^n$ ligands are coordinated to copper(I) cyanoacetate and cyanomethylcopper(I), respectively, forming coordinatively saturated complexes.[5] Determination of the number of the phosphine ligand coordinated to the copper complexes involved in the reversible decarboxylation by isolating the copper–phosphine complexes is impossible, because NCCH$_2$CO$_2$Cu·(PBu$_3{}^n$)$_x$ (x = 1, 2, and 3) is too unstable toward decarboxylation at ambient temperature to permit its isolation. General expressions NCCH$_2$CO$_2$Cu·(PBu$_3{}^n$)$_x$ and