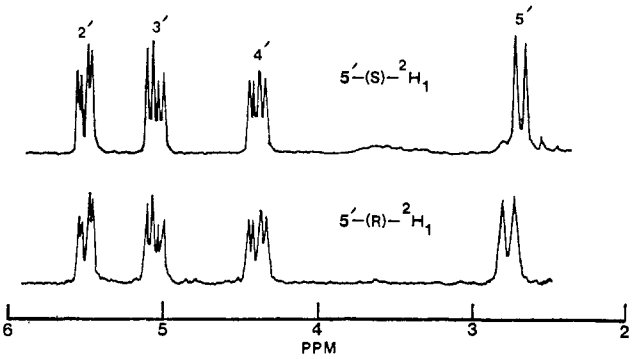

Figure 1. 90-MHz ^1H NMR spectra ($CDCl_3$) of [5'-$^2H_1$]-2',3'-*O*-isopropylidene-5'-deoxy-5'-(methylthio)adenosine.

of the benzoyl protecting groups from **6** and **7** then produced the desired reference compounds **8** and **9**. The 90-MHz proton NMR spectra of these two compounds are shown in Figure 1. It is clear from these spectra that the two diastereomerically deuterated compounds can be readily differentiated.

The synthesis of the reference compounds having been accomplished, attention was turned to the synthesis of [5'(*R*)- and [5'(*S*)-$^2H_1$]ATP. The chirally deuterated N^6-benzoyl derivatives **4** and **5** were deprotected[2] to yield [5'(*R*)- and [5'(*S*)-$^2H_1$]-2',3'-*O*-isopropylideneadenosine, and these two chirally labeled nucleosides were then converted to [5'(*R*)- and [5'(*S*)-$^2H_1$]-adenosine monophosphate (AMP) via their cyanoethylphosphate derivatives.[6] The configuration at C-5' of the adenosine nucleus is unaltered by this reaction sequence since the formation of the intermediate cyanoethylphosphate esters proceeds by attack of the alcoholic hydroxy group of the nucleoside upon an activated phosphate ester generated from cyanoethylphosphate anion and DCC.[7] The two forms of chirally deuterated AMP so obtained were transformed into [5'(*R*)- and [5'(*S*)-$^2H_1$]ATP by reaction of their morpholidate derivatives with tri-*n*-butylammonium pyrophosphate.[6]

The stereochemistry of SAM formation was elucidated by incubating the two chirally deuterated forms of ATP with a partially purified preparation of methionine adenosyltransferase obtained from dried yeast by the method of Chiang and Cantoni.[8] The two samples of chirally deuterated SAM produced by the enzyme were each isolated as their phosphotungstate derivatives.[9] After removal of the phosphotungstate ion,[9] the partially purified samples of SAM were hydrolyzed with boiling water[10] to yield [5'(*S*)- and [5'(*R*)-$^2H_1$]-5'-deoxy-5'-(methylthio)adenosine (**10**, **11**) (Scheme III). Final purification of **10** and **11** was accomplished by conversion[11] to [5'-(*S*)- and [5'(*R*)-$^2H_1$]-2',3'-*O*-isopropylidene-5'-deoxy-5'-(methylthio)adenosine (**8**, **9**) followed by chromatography.

The chirality of the labels in the two enzymatically derived compounds **8** and **9** was determined by comparison of their proton NMR spectra with the NMR spectra of the synthetically prepared reference substances. The results of this comparison were as follows: the SAM derived from [5'(*S*)-$^2H_1$]ATP yielded [5'-(*R*)-$^2H_1$]-2',3'-*O*-isopropylidene-5'-deoxy-5'-(methylthio)adenosine (**9**) while the SAM produced from [5'(*R*)-$^2H_1$]ATP yielded the 5'-(*S*) compound **8**. Therefore, one can conclude that the formation of *S*-adenosylmethionine by yeast methionine adenosyltransferase takes place with *inversion* of configuration at C-5' of ATP. These results support a single displacement mechanism (Scheme IIA) for SAM formation.

Acknowledgment. We would like to thank Professor Frederick B. Rudolph for helpful advice concerning enzyme purification and the National Institutes of Health (GM-26166) and the Robert A. Welch Foundation (C-729) for financial support.

Registry No. 1, 63-68-3; (*R*)-**2**, 80375-30-0; (*S*)-**2**, 80408-88-4; **8**, 80375-31-1; **9**, 80408-89-5; **10**, 80375-32-2; **11**, 80408-90-8; methionine adenosyltransferase, 9012-52-6.

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A Novel and Efficient Entry to (±)-Quadrone†

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Quadrone (**1**), a fungal metabolite from *Aspergillus terreus* reported in 1978, was found to exhibit inhibitory activity in vitro against human epidermoid carcinoma of the nasopharynx (KB) with an ED_{50} of 1.3 μg/mL and in vivo against P338 lymphocytic leukemia in mice.[1] The reported biological activity and the deceptively challenging structural features of this novel sesquiterpene have combined to trigger intensive efforts directed at total synthesis. Two independent entries to quadrone sharing a general strategic theme have recently been reported by Danishefsky[2] and Helquist.[3] We describe herein our efforts directed along an entirely different tactical protocol, culminating in a direct and efficient construction of the quadrone nucleus and a formal total synthesis.

In evaluating quadrone as a target for total synthesis, attention is quickly drawn to the five contiguous asymmetric centers decorating the quadricyclic framework. At a more subtle level lies the recognition that the centers of asymmetry are shared over the structure such that the four rings have four, three, three, and three chiral centers, respectively. We focused on the quaternary carbon [C(1) in **1**] as the only center of asymmetry common to each of the four rings of the natural product. It was felt that this quaternary center should be established at the outset in a synthetic precursor to quadrone, with surrounding functionality ripe for elaboration. The spiro[4.5]decadienone **2**, readily available in 64% overall yield from 2-methyldimedone isobutyl ether (**3**),[4] fulfilled these requirements as a masked quadrone synthon.

The critical transmutation of the spiro[4.5]decadienone **2** into the tricyclic enedione **5** afforded an interesting study of site-selective reactivity in a polyfunctional molecule (Scheme I). Oxidative cleavage of the trisubstituted olefin linkage in **2** [OsO_4(catalytic), *N*-methylmorpholine *N*-oxide,[5] 1:1 H_2O/acetone; $NaIO_4$ (8 equiv), 1:1 H_2O/THF] proceeded cleanly to give the doubly appended cyclopentenone **4** in 94% yield.[6] Note that in **4a** there are highlighted three potential nucleophilic sites

† This paper is dedicated to Professor Gilbert Stork on the occasion of his 60th birthday.

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(6) The doubly appended cyclopentenone **4** is presented in three different conformational perspectives (**4a-c**) to focus on each of three possible electrophile-nucleophile pairings.

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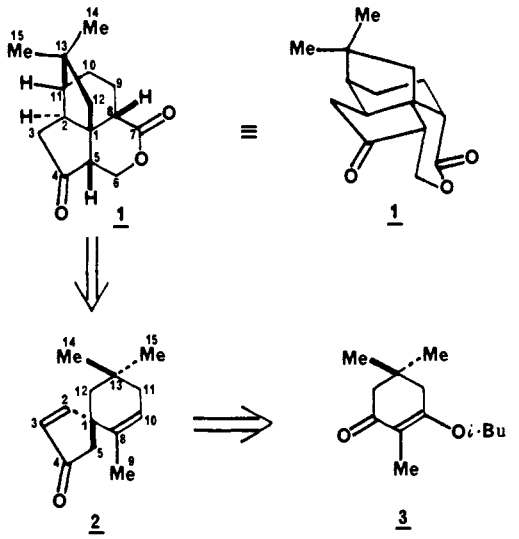


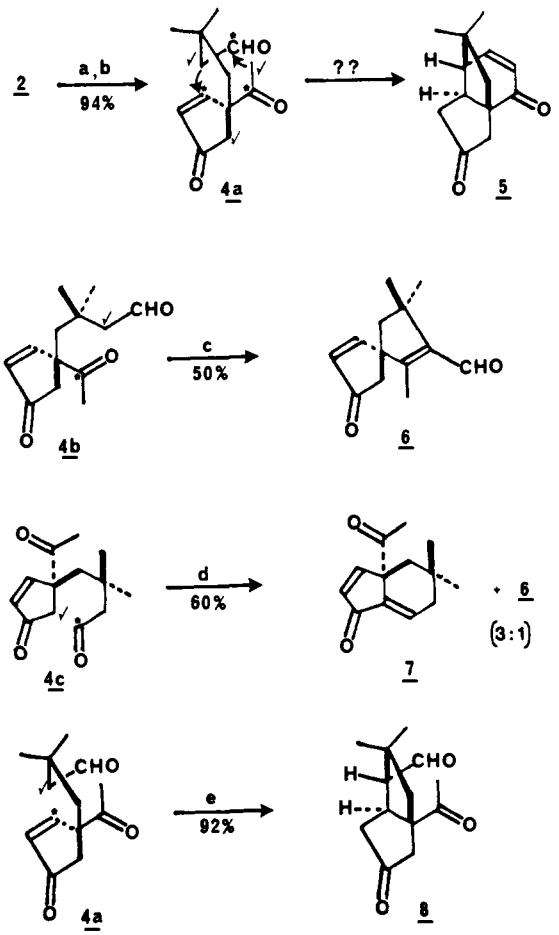
(√) and three potential electrophilic sites (*). The elaboration of **4** to **5** required a specific double pairing of electrophile–nucleophile conjugates, as indicated. In the face of other quite reasonable possibilities, this was clearly a demanding requirement, with selective reaction via the biased conformation **4a** far from assured.

In the event, the geminally substituted cyclopentenone **4** exhibited reactivity along *each* of three pathways, *to the near or complete exclusion of the other two* (Scheme I).[7] Treatment of **4** with $TiCl_4$ followed by *N*-methylanilinium trifluoroacetate (TAMA)[8] (−35 → 25 °C) gave as the sole isolable product the spiro[4.4]bicyclic **6** (mp 108–111 °C) in 50% yield, reflecting reactivity through conformation **4b**. The dienedione **7** (mp 65–67 °C) was the major product together with **6** (**7**/**6** = 3:1) when **4** was heated at reflux in benzene with a catalytic amount of *p*-toluenesulfonic acid, resulting from the electrophile–nucleophile pairing shown in **4c**. The key observation was that the desired Michael addition, **4a** → **8** (mp 68–70 °C) could be effected *in 92% yield* by heating a solution of **4** in benzene at reflux together with 2 equiv of morpholine and a catalytic amount of *p*-toluenesulfonic acid.

The establishment of the tricarbocyclic nucleus of quadrone and the functional manipulation upon that framework are detailed in Scheme II. After many fruitless attempts at effecting the aldol closure of **8** to **5** (a closure across the convex face of a cis-fused bicyclo[3.3.0]octane system), we found that finely powdered NaOH (1.1 equiv) and dibenzo-18-crown-6 (0.6 equiv) in benzene at reflux converted **8** cleanly to **5** (mp 59–61 °C) *in 96% yield*.

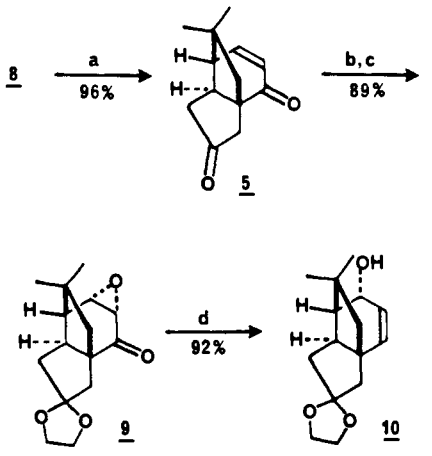
With the tricyclic enedione **5** in hand, the major obstacle remaining was the formation of the C(7)–C(8) bond (see **1**) in the contrathermodynamic (axial) α orientation.[9] Toward this end, **5** was ketalized under standard conditions (93%) and epoxidized (*t*-BuOOH, NaOH, MeOH, H_2O, 25 °C)[10] to provide the α,β-epoxyketone **9** (mp 91-92 °C) in 89% overall yield. Treatment of **9** with hydrazine hydrate in MeOH/HOAc (−78 → 25 °C) gave the Wharton reaction[11] product **10** (mp 69–71 °C) in 92% yield. The α-oriented hydroxyl group at the incipient C(10) position was thus situated to exercise remote control over the stereochemistry of the C(7)–C(8) bond via intramolecular delivery. It should be noted that the yields in the sequence **2** → **10** were uniformly excellent (*69% overall*) and that all intermediates were nicely crystalline.

Scheme I[a]



[a] (a) OsO_4 (catalytic), *N*-methylmorpholine *N*-oxide, 1:1 acetone/H_2O. (b) $NaIO_4$ (8 equiv), 1:1 THF/H_2O. (c) $TiCl_4$, TAMA,[8] THF, −35 → room temperature. (d) *p*-TsOH (catalytic), PhH, reflux. (e) morpholine (2 equiv), *p*-TsOH, PhH, reflux.

Scheme II[a]



[a] (a) NaOH (powdered, 1.1 equiv), dibenzo-18-crown-6 (0.6 equiv), PhH, reflux. (b) ethylene glycol (1.6 equiv), *p*-TsOH (catalytic), PhH, reflux. (c) *t*-BuOOH, NaOH, MeOH, H_2O, 25 °C. (d) H_2NNH_2, MeOH, HOAc.

The requisite α-oriented carbon appendage at C(8) was introduced with complete stereocontrol by application of a standard Claisen rearrangement sequence[12] to the allylic alcohol **10**.

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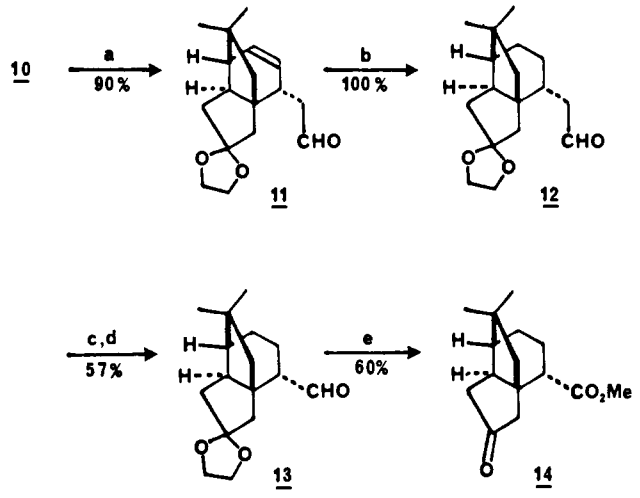
Scheme III[a]



[a] (a) Ethyl vinyl ether, $Hg(OAc)_2$; 250 °C, *o*-xylene, sealed tube. (b) H_2 (1 atm), 5% Pd/C, EtOH, 25 °C. (c) Ac_2O, KOAc, reflux. (d) OsO_4 (catalytic), $NaIO_4$ (8 equiv), 1:1 THF/H_2O; (e) Jones reagent; CH_2N_2, Et_2O.

Treatment of **10** with ethyl vinyl ether in the presence of mercuric acetate followed by heating the resultant allyl vinyl ether at 250 °C in a sealed tube in *o*-xylene afforded the γ,δ-unsaturated aldehyde **11** in 90% yield (56% conversion) (Scheme III).

With the critical C(8) stereocenter thus established, the formal total synthesis of (±)-quadrone followed directly. Catalytic hydrogenation [H_2 (1 atm), 5% Pd/C, EtOH] of **11** provided **12** in 100% yield. A one-carbon degradation of the aldehyde was then effected by enol acetylation (Ac_2O, KOAc, reflux)[13] followed by treatment with a catalytic amount of OsO_4 and $NaIO_4$ (8 eq) in 1:1 THF/H_2O at 25 °C.[14] The resultant aldehyde **13** was subjected to Jones oxidation and esterification (CH_2N_2, Et_2O, 0 °C → room temperature) to generate the keto ester **14** (mp 47–49 °C; lit.[2] 49–51 °C). Compound **14** proved to be identical by IR spectroscopy, MS, TLC, and 400-MHz ^1H NMR spectroscopy to a sample kindly provided by Professor Danishefsky.

Since **14** has been converted to (±)-quadrone (**1**),[2] the formal total synthesis was then complete. The transformation of the spiro[4.5]decadienone **2** to the keto ester **14** was accomplished in 21% overall yield. Further refinements of this scheme and efforts directed at a *de novo* lactone assembly (**12** → **1**) will be reported with full details in due course.[15]

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Registry No. (±)-**1**, 74807-65-1; (±)-**2**, 80359-35-9; (±)-**4**, 80359-36-0; (±)-**5**, 80375-22-0; (±)-**6**, 80359-37-1; (±)-**7**, 80359-38-2; (±)-**8**, 80387-00-4; (±)-**9**, 80359-39-3; (±)-**10**, 80359-40-6; (±)-**11**, 80359-41-7; (±)-**12**, 80359-42-8; (±)-**13**, 80359-43-9; (±)-**14**, 78739-62-5.

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(15) The structural assignments of all compounds reported herein are fully supported by IR, MS, ^{13}C NMR, 400-MHz ^1H NMR data and elemental analysis.

Microsomal Reduction of the Carcinogen Chromate Produces Chromium(V)

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The metabolism of procarcinogens to reactive intermediates by cellular enzymes has been shown to be a critical step in the ability of many organic chemicals to react with nucleic acids and proteins in vivo and initiate cancer.[1] The importance of this process in carcinogenesis by inorganic chemicals has been unknown. We have previously reported that rat liver microsomes in the presence of NADPH enzymatically reduced chromate to chromium(III)[2] and that the electron-transport cytochrome P-450 system was responsible for the chromate-reductase activity of microsomes.[3] We now report that a stable reactive intermediate, chromium(V), is formed upon metabolism of the inorganic carcinogen chromate by rat liver microsomes in the presence of NADPH.

Incubation of chromate with rat liver microsomes and NADPH in 0.05 M Tris·HCl, pH 7.4, resulted in the appearance of an EPR signal with $g = 1.979$ and $\Delta H = 8.5$ G (Figure 1). No EPR signal was detected upon incubation of chromate with microsomes in the absence of NADPH. A 10- to 20-fold weaker signal was seen when chromate was incubated with NADPH in the absence of microsomes. The intensity of the signal increased with increasing concentration of microsomal protein. Peak intensity of the signal was optimized at a NADPH concentration of 0.85 mM by using a microsomal protein concentration of 11.6 mg/mL.

The EPR signal characteristic of chromium(V) appeared within 20 s after initiating the reaction of chromate with microsomes and NADPH at 22 °C. The intensity of the signal decreased rapidly in the first 2 min, increased slightly after 5 min, and then slowly decayed after 15 min (Figure 2). The signal persisted 80 min after initiating the reaction. The EPR signal observed 10 min after initiating the reaction was asymmetric (Figure 1) with a shoulder to lower field of the main peak. Power saturation studies revealed that this shoulder represented a distinct signal since it saturated at lower powers than the major $g = 1.979$ peak. This shoulder was absent in the 30-s sample. The appearance of the lower field signal at later times may account for the slight increase in peak intensity seen between 5 and 15 min (Figure 2).

Stable chromium(V) intermediates with $g \sim 1.98$ and $\Delta H =$ 2–20 G have been observed during the oxidation of organic acids, alcohols, and thiols.[4-9] EPR signals with $g \sim 1.98$ and $\Delta H =$ 7–17 G were detected in breast, liver, and thyroid tissues which had been incubated with solutions containing chromium(VI).[10] The present data indicate that the complete rat liver microsomal system is capable of producing two chromium(V) species upon metabolism of chromate. A direct one-electron transfer from the microsomal electron-transport cytochrome P-450 system to

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