extracted with ether and the combined extracts washed with water, dried, and evaporated under vacuum to give 3.1 g of crude keto phosphonamide **11** as an oil. The nmr spectrum ($CDCl_3$) showed multiplets at 0.9, 1.3, 1.6, and 3.2 and a doublet at 2.62 (J = 10) ppm.

Preparation of β-Ketophosphonic Acid Bis(dimethylamide) (9) by Oxidation of the Corresponding β-Hydroxy Adduct. A stirred solution of 0.100 g (0.39 mmol) of β-hydroxyphosphonic acid bis(dimethylamide) (**3**, $R_1 = C_6H_5$; $R_2 = R_3 = H$; $R_4 = CH_3$) and 0.5 g of activated manganese dioxide (excess) in 4 ml of $CHCl_3$ was heated at reflux for 10 min. The manganese dioxide was then removed by filtration, and the solution was evaporated to give 90 mg (90%) of keto phosphonamide **9**, 97% pure by nmr analysis.

Reduction of β-Ketophosphonic Acid Bis(dimethylamide) (9). Synthesis of *trans*-1-Phenylpropene. To a stirred solution of 0.120 g (0.473 mmol) of β-ketophosphonic acid bis(dimethylamide) (**9**) in 3 ml of methanol was slowly added, while at 0°, 20 mg (excess) of sodium borohydride. After 1 hr of stirring, dilute hydrochloric acid was added to decompose the excess sodium borohydride, and the product was isolated by extraction with ether. The ether extracts were washed with water, dried, and evaporated under vacuum to afford 0.096 g (80%) of β-hydroxy phosphonamide **3** ($R_1 = C_6H_5$; $R_2 = R_3 = H$; $R_4 = CH_3$).

The crude product from above was heated at reflux in 3 ml of toluene containing 0.3 g of silica gel for 15 hr. Vpc analysis showed that *trans*-1-phenylpropene was produced in 98% purity. Using bromobenzene as an internal standard, the yield was estimated to be 92%.

Reduction of Ketophosphonic Acid Bis(dimethylamide) (11). Synthesis of *trans*-1-*t*-Butyl-1-nonene. The procedure of Eliel[13] for reductions using lithium aluminum hydride and aluminum chloride was followed. To a mixture prepared by stirring 0.402 g (3.0 mmol) of aluminum chloride and 0.032 g (0.86 mmol) of lithium aluminum hydride in 12 ml of ether for 0.5 hr at room temperature was added 0.600 g of a 3:1 mixture of β-keto phosphonamide **11** and octylphosphonic acid bis(dimethylamide). The resulting mixture was stirred for 2.5 hr and 0.5 ml of *t*-butyl alcohol then added to decompose the excess reagent. After 1 hr, 0.025 g more of the 3:1 mixture was added and the solution stirred for 30 hr at room temperature. Water was added, and the products were isolated by ether extraction. The ether extracts were washed with water, dried, and evaporated to give 0.432 g of a clear oil containing a trace of keto phosphonamide **11**, 5–10% *trans*-1-*t*-butylnonene (possibly formed during isolation), octylphosphonic acid bis(dimethylamide), and β-hydroxy phosphonamide **3** (R_1 = *t*-butyl; $R_2 = R_3 = H$; $R_4 = C_7H_{15}$).

Decomposition of 0.154 g of the above oil in refluxing toluene containing 0.3 g of silica gel gave a 94:6 ratio of *trans*- and *cis*-1-*t*-butylnonenes. Thermolysis of 0.250 g of the above product in refluxing benzene for 1.75 hr afforded pure *trans*-1-*t*-butylnonene in 71% yield by vpc analysis.

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Total Synthesis of Sativene

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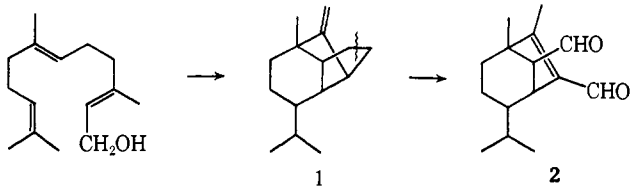
Contribution from the Division of Natural Sciences, University of California, Santa Cruz, California 95060. *Received May 24, 1968*

Abstract: A stereospecific total synthesis of the tricyclic sesquiterpene hydrocarbon, sativene (**1**), is described, and several interesting reactions are developed. A useful extension of the Grignard reaction is introduced whereby enolization can be suppressed in favor of addition by conducting the reaction at low temperature. A new method of protecting carbonyl groups toward hydroboration by formation of the 2,4-DNP derivative is reported, and an intramolecular alkylation [**16** → **17**] to construct the tricyclic carbon skeleton is described.

In a noteworthy display of chemical intuition, de Mayo, in 1962, suggested a pathway for the biogenesis of the then recently isolated sesquiterpene, helminthosporal, the toxin form *Helminthosporium sativum*.[1] The proposed pathway proceeded from farnesol *via* a relatively straightforward cyclization to the tricyclic sesquiterpene hydrocarbon **1**. **1** was then assumed to undergo oxidative cleavage of the indicated carbon–carbon bond to give helminthosporal (**2**).



1 2

At the time this prediction was made however, the carbon skeleton of **1** was unknown in sesquiterpene chemistry. In 1965, de Mayo and Williams provided strong support for this pathway when they were able to isolate small amounts of a sesquiterpene hydrocarbon, sativene, from *Helminthosporium sativum*, and to show that sativene did indeed possess the predicted structure, **1**.[2] We wish now to report the first, completely stereospecific, total synthesis of this unique sesquiterpene and to record a general method of entry into the tricyclic carbon skeleton.

Discussion and Results

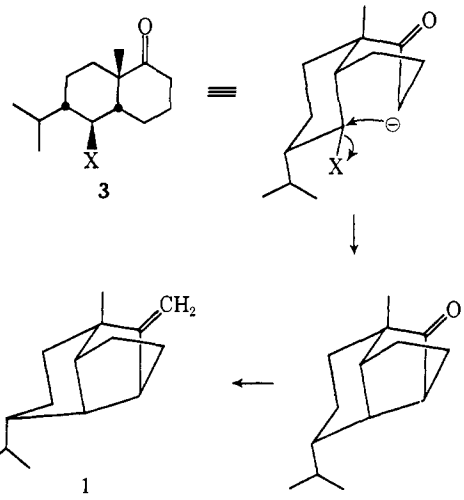
An examination of Dreiding models of *cis*-decalin reveals that when ring B is in a boat conformation carbons **1** and **6** are quite close. Thus we considered that an interesting method for forming the ring system of sativene would be to synthesize a molecule such as **3** (where X = any leaving group) and to see if the requisite bond could be formed by an intramolecular alkylation.[3]

There are some interesting stereochemical problems associated with the synthesis of **3** (X = OTs) which

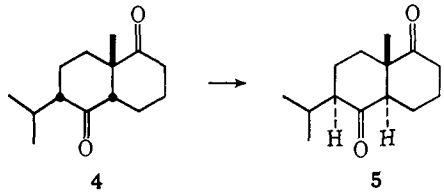
(1) P. de Mayo, R. Robinson, E. Y. Spencer, and R. W. White, *Experientia*, **18**, 359 (1962).

(2) P. de Mayo and R. E. Williams, *J. Am. Chem. Soc.*, **87**, 3275 (1965).

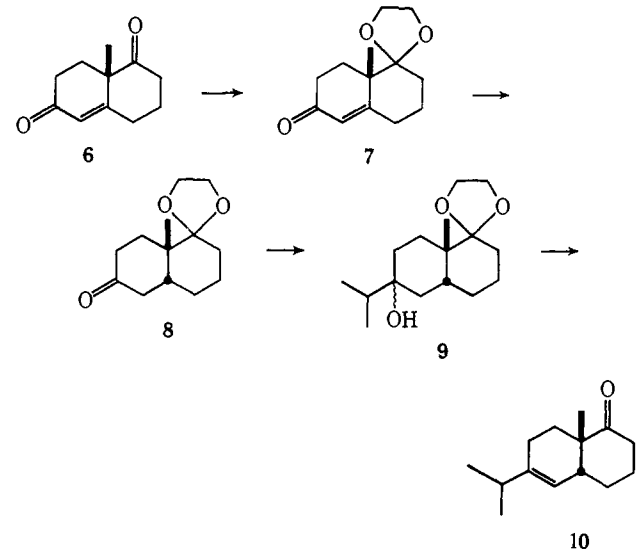
(3) There are several reports in the literature of various successful intramolecular alkylations, for example the notable synthesis of copaene: C. H. Heathcock, R. A. Badger, and J. W. Patterson, *ibid.*, **89**, 4133 (1967). No case similar to that reported here has been published however.



should be briefly commented upon. It should be noted for example that this *cis*-fused decalin is not the most stable of the possible stereoisomers of **3**. One would expect the *trans*-fused isomer to be the more stable. This implies that **3** could probably not be made from any precursor in which C_1 was ketonic. For instance, in **4** both the isopropyl group and the fused ring should readily epimerize to give **5**, a compound useless for our purposes. Synthetically, therefore, one needs to establish all of the four centers of asymmetry by stereospecific, kinetically controlled reactions, which do not necessarily lead to the more stable product.



This was accomplished in the following manner. Corey has shown[4] that the Wieland–Miescher ketone **6** can be selectively ketalized at the saturated carbonyl group, and this monoketal **7** was chosen as starting material. By carefully controlling the reaction conditions, the yield of monoketal, mp 66–67°, was consistently >90%. *cis* fusion of the decalin ring system was then readily established by catalytic hydrogenation of the enone **7** at atmospheric pressure over palladized charcoal. The product **8** mp 54–54.5°, was isolated in 90% yield. Assignment of *cis* stereochemistry to the product was based on a wealth of precedent,[5] and upon nmr studies of the line width at half-height of the angular methyl resonance.[6] This method of assigning stereochemistry to *cis*/*trans* decalins is based on the fact that the angular methyl group in *trans*-decalins shows a slightly broader resonance line than in the corresponding *cis* compound, and seems to be on sufficiently firm ground that it is of predictive value when only one of the two isomers is available. Thus, using tetramethylsilane as reference, $\Delta W_{h/2} = 0.37$ Hz for compound **8**, definitely in the *cis* range of values. One would expect[6] $\Delta W_{h/2}$ to be on the order of 0.6–0.8 Hz for the corresponding *trans* isomer. In addition to the *cis* product, nmr revealed the presence of approximately 5% of a second product, presumed to be the *trans*-fused isomer.

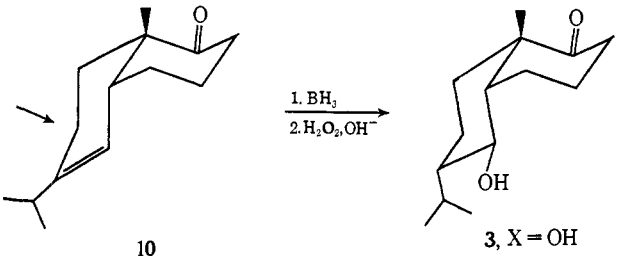


Treatment of **8** with isopropylmagnesium bromide in refluxing ether led, as expected,[7] to only a minute (5%) yield of tertiary alcohol **9**. Enolization of the carbonyl appears to be the main course of this reaction since much starting material is recovered. On further experimentation however we discovered that, remarkably enough, when the Grignard reaction was carried out at −50° for several hours, a 50% yield of tertiary alcohol **9** was obtained along with recovered starting material as judged by nmr. Upon stirring the crude product mixture overnight in a two-phase system of hexane–50% aqueous sulfuric acid, a 45% yield of olefinic ketone **10** could be obtained. Although there are indications in the literature[8] that, in Grignard reactions, addition increases at the expense of enolization as the temperature is lowered from 35 to 0°, the magnitude of the effect observed here is unprecedented. We are currently engaged in a systematic study of the scope of this temperature effect, and preliminary results indicate that this useful modification of the Grignard reaction is quite general. When **8** was subjected to several successive treatments with isopropylmagnesium bromide at −50°, followed by acidic dehydration and deketalization, a 70% yield of **10** (2,4-DNP, mp 122–123°) was obtained. Alternatively, reaction of **8** with isopropyllithium in refluxing pentane gave an approximately 60% yield of alcohol **9**. Several successive treatments followed by acid hydrolysis and dehydration produced **10** in 80% yield.

That the dehydration of **9** had indeed occurred in the desired direction to give **10** follows from the nmr spectrum of the product (τ 4.90, 1 H singlet). The vinyl proton is unsplit by the neighboring proton at the ring junction, for, as measured from Dreiding models, the dihedral angle between the two protons is approximately 80°.[9]

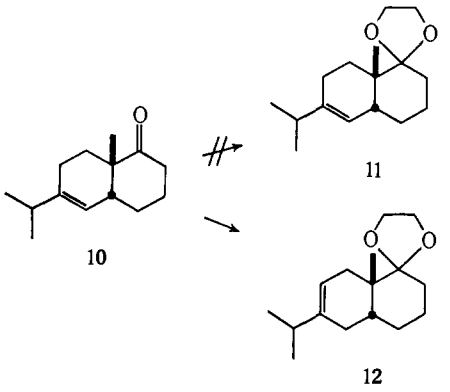
(4) E. J. Corey, M. Ohno, R. B. Mitra, and P. A. Vatakencherry, *J. Am. Chem. Soc.*, **86**, 478 (1964).
(5) C. B. C. Boyce and J. S. Whitehurst, *J. Chem. Soc.*, 2680 (1960).
(6) K. L. Williamson, T. Howell, and T. A. Spencer, *J. Am. Chem. Soc.*, **88**, 325 (1966).
(7) E. P. Kohler and D. Thompson, *ibid.*, **55**, 3822, (1933).
(8) V. Grignard and Blanchon, *Bull. Soc. Chim. France*, [4] **49**, 23 (1931).
(9) M. Karplus, *J. Chem. Phys.*, **30**, 11, (1959).

Our original synthetic plan called for the establishment of the two remaining asymmetric centers by hydroboration–basic-peroxide oxidation of the double bond in **10**. Attack would be expected from the convex face of the *cis*-decalin to produce **3** (X = OH).



10 **3**, X = OH

Attempted hydroboration with 0.5 equiv of borine in THF resulted in preferential reduction of the carbonyl, rather than of the olefinic double bond. When, however, the carbonyl was ketalized for protection (benzene–toluenesulfonic acid method) a migration of the double bond from $\Delta^{1,2}$ to $\Delta^{2,3}$ also occurred [**10** → **12**] rendering the ketal useless. The assignment again follows from the nmr of the product (τ 4.70, 1 H broad triplet). Several ketalization experiments were carried out using weaker acid catalysts (*e.g.*, oxalic acid) in the hope that bond migration might be made much slower than ketalization, but to no avail.



10 **11** **12**

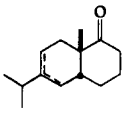
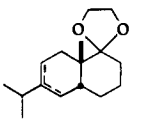
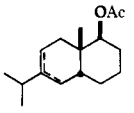
Steric reasons for double-bond positions in such octalins are far from clear[10] and prolonged inspection of Dreiding models yields no ready explanation for the observed preferences in this case. In any event, the positional preference of the double bond is not great (see Table I below) indicating that there are only small energy differences involved. It is possible that subtle changes in bond angles[11] throughout the molecule on changing C_5 from trigonal to tetrahedral hydridization perturb the severity of various steric interactions and are responsible for the double-bond shift. In support of this argument, the following equilibrium positions of the double bond were established as estimated from nmr. In both cases where C_5 is tetrahedral, the double bond is mostly stable $\Delta^{2,3}$. In the case where C_5 is trigonal, the double bond is primarily $\Delta^{1,2}$.

By this reasoning, it thus appears that in order for the double bond to remain $\Delta^{1,2}$, the carbonyl function should be protected toward hydroboration in some manner which allows C_5 to remain trigonal.

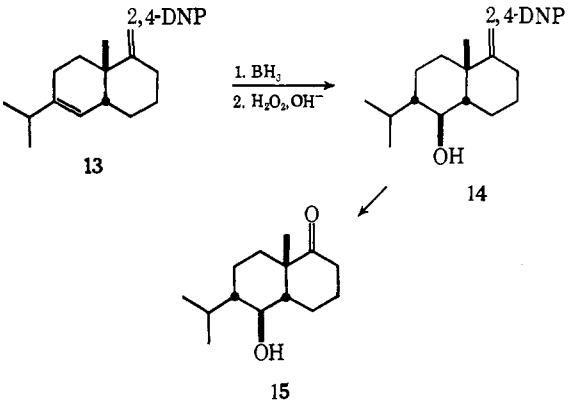
(10) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 276.

(11) Similar conclusions are reached by Corey and Sneen in their vector analysis of $\Delta^{1,2}$ *vs.* $\Delta^{2,3}$ octalins: E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, 77, 2505 (1955).

Table I

	$\Delta^{1,2}$, %	$\Delta^{2,3}$, %
	85	15
	<10	>90
	30	70

Oximes[12] and oxime acetates[13] are possibilities, but these are known to be attacked by BH_3. The reaction of borine with hydrazones, however, has not been reported. We found[14] that when 2,4-dinitrophenylhydrazone (**13**), prepared in 80% yield from **10**, was treated with 0.5 equiv of BH_3 in THF under the usual conditions,[15] and the resulting dialkylborane intermediate was oxidized by basic peroxide, that the 2,4-DNP alcohol **14**, mp 196°, was produced as the sole product in 65% yield.



13 **14** **15**

Removal of the 2,4-DNP protecting group was readily accomplished by ozonolysis in ethyl acetate at −78°,[16] and after a reductive work-up with sodium bisulfite, alcohol **15** was isolated in 70% yield. That no unexpected changes occurred during the ozonolysis was demonstrated by reconverting **15** into its 2,4-DNP, mp 196°, and verifying the identity of the product with **14**.

At this point, the four required centers of asymmetry had been cleanly established, and there remained only the intramolecular alkylation. The tosylate **16** was readily prepared by reaction of ketol **15** with tosyl chloride in pyridine at room temperature for 3 days. Treatment of this keto tosylate, mp 146°, with 1 equiv of dimethylsulfinyl carbanion in DMSO[17] at 60°

(12) H. Feuer, B. F. Vincent, Jr., and R. S. Bartlett, *J. Org. Chem.*, **30**, 2877 (1965).

(13) A. Hassner and P. Catsoulacos, *Chem. Commun.*, 590 (1967).

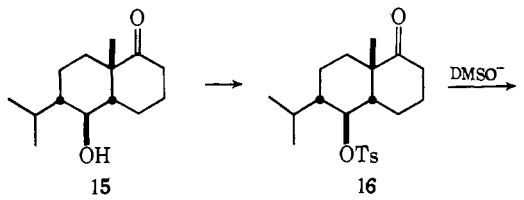
(14) A preliminary report of this reaction has already been published: J. E. McMurry, *ibid.*, 433 (1968).

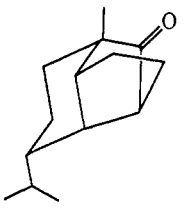
(15) H. C. Brown and B. C. Subba Rao, *J. Am. Chem. Soc.*, **81**, 6423, 6428 (1959).

(16) According to the procedure of G. A. Fleisher and E. C. Kendall, *J. Org. Chem.*, **16**, 556 (1951).

(17) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **87**, 1345 (1965).

for 2 hr gave, in 90% yield, the tricyclic ketone **17**, showing ir absorption at 1745 cm^{-1} (cyclopentanone carbonyl).





17

Completion of the synthesis by conversion of **17** into *dl*-sativene was readily accomplished for, although **17** was completely inert to methylenetriphenylphosphorane in DMSO,[18] treatment with methyllithium followed by dehydration of the resulting tertiary alcohol with thionyl chloride in pyridine yielded *dl*-sativene (**1**).

Synthetic *dl*-sativene was identical with the natural material by spectral criteria (ir and nmr), and gave a suitable mass spectrum (P^+ *m/e* 204). Unfortunately no direct comparison was possible due to the lack of an authentic sample.

Experimental Section

Preparation of the Monoketal of the Wieland–Miescher Ketone, 7. The Wieland–Miescher ketone (10.0 g, 0.056 mol) was dissolved in 260 ml of benzene. Ethylene glycol (35 ml) and 100 ml of *p*-toluenesulfonic acid monohydrate were added, and the mixture was brought to reflux in a flask fitted with a Dean–Stark trap. The mixture was refluxed for 25 min until 0.9 equiv (0.9 ml) of water was collected. Solid sodium bicarbonate was added; the reaction was cooled and diluted with water and the benzene layer drawn off. The aqueous layer was further extracted with ether. The organic layers were dried ($MgSO_4$), filtered, and evaporated to yield 12.0 g (97%) of a clear colorless oil. Nmr of the crude product revealed it to be a mixture of 90% desired product, 7% starting material, and 3% diketal. The crude product could either be used directly in the next step, or, if desired, placed in the refrigerator for crystallization. Recrystallization from isopropyl ether gave material, mp 66–67° (lit.[4] 66–67°).

Hydrogenation of Enone 7 to *cis*-Ketone 8. The monoketal 7 (12.0 g, 0.054 mol) was dissolved in 50 ml of 95% ethanol, and 1.0 g of 10% palladium-on-carbon catalyst was added. Hydrogenation occurred smoothly at atmospheric pressure and was complete in 45 min. The solution was then filtered free of catalyst, and the catalyst was washed with 95% ethanol. The filtrates were combined and evaporated to yield 12.0 g of crude product. Nmr of this crude material showed two compounds to be present in the ratio 95:5 judging from the relative integrated intensities of the angular methyl protons. On standing in the refrigerator, the major product crystallized. Recrystallization from isopropyl ether gave the analytical sample: mp 54–54.5°; yield 10.8 g (90%); ir (KBr) 1710 cm^{-1} (C=O); nmr ($CDCl_3$) τ 6.05 (s, 4 H) and 8.80 (s, 3 H).

Anal. Calcd for $C_{13}H_{20}O_3$: C, 69.61; H, 8.99. Found: C, 69.85; H, 9.13.

That this was indeed the *cis*-fused compound was confirmed by measurements of the line width at half-height of the angular methyl resonance in the nmr.[6] Using TMS as the reference, the following values were found: $W_{h/2\,TMS}$ = 0.45 Hz; $W_{h/2\,(8)}$ = 0.82 Hz; $\Delta W_{h/2}$ = 0.37 Hz, clearly *cis*.

2-Isopropyl-4aβ-methyl-3,4,4a,7,8,8aβ-hexahydronaphthalen-5-(6H)-one (10). Ketone **8** (2.2 g, 0.010 mol) was dissolved in 20 ml of ether and added to 100 ml of a 0.5 *M* solution of isopropylmagnesium bromide in ether at −50°. The reaction was stirred for 3 hr at −50°, then quenched by addition of 10% aqueous sulfuric acid. The ether solution was drawn off, dried ($MgSO_4$), filtered, and evaporated. The crude product was dissolved in 20 ml of ether and treated once again with isopropylmagnesium bromide as described above. After three such treatments, the crude product was dissolved in 100 ml of hexane. Aqueous sulfuric acid (100 ml, 50%) was added, and the reaction was stirred overnight at room temperature. The hexane layer was drawn off, dried ($MgSO_4$), filtered, and evaporated. Chromatography of the crude oily product over alumina (elution with 1:1 hexane–ether) gave 1.45 g (70%) of the olefinic ketone **10**.

Alternatively, **10** could be prepared in the following manner. Ketone **8** (2.2 g, 0.010 mol) in 20 ml of hexane was added to 30 ml of a 2 *M* solution of isopropyllithium in pentane. The solution was refluxed for 1 hr, then quenched with water. The organic layer was drawn off, dried ($MgSO_4$), filtered, and evaporated. After three such treatments, the product was submitted to the hydrolysis–dehydration conditions described above; 1.65 g (80%) of product was obtained as a clear, colorless oil: nmr (CCl_4) τ 4.90 (s, 1 H), 8.92 (s, 3 H), and 9.08 (d, 6 H, J = 7 Hz).

2-Isopropyl-4aβ-methyl-3,4,4a,7,8,8aβ-hexahydronaphthalen-5-(6H)-one, 2,4-Dinitrophenylhydrazone (13). A solution[19] of 2,4-dinitrophenylhydrazine was made by dissolving 8.0 g (0.041 mol) of the reagent in 30 ml of concentrated sulfuric acid and adding 50 ml of water and 150 ml of 95% ethanol. To this solution was added ketone **10** (7.3 g, 0.035 mol) dissolved in 30 ml of methanol. After 10 min at room temperature, the reaction mixture was filtered and the product collected. Several recrystallizations from ethanol gave the analytical sample: mp 122–123°; yield 10.8 g (80%); ir ($CHCl_3$) 3350, 1590, 1610, and 1500 cm^{-1}; nmr ($CDCl_3$) τ 0.90 (d, 1 H, J = 2 Hz), 1.72 (doublet of doublets, 1 H, J = 10 Hz, J' = 2 Hz), 2.10 (d, 1 H, J = 10 Hz), 4.82 (s, 1 H), 8.62 (s, 3 H), and 8.98 (d, 6 H, J = 7 Hz).

Anal. Calcd for $C_{20}H_{26}N_4O_4$: C, 62.16; H, 6.78. Found: C, 62.23; H, 6.72.

2α-Isopropyl-1β-hydroxy-4aβ-methyl-1,2,3,4,4a,7,8,8aβ-octahydronaphthalen-5(6H)-one, 2,4-Dinitrophenylhydrazone (14). Olefinic 2,4-dinitrophenylhydrazone **13** (20.0 g, 0.026 mol) was dissolved in 140 ml of THF under nitrogen. A 1 *M* solution (15 ml) of BH_3 in THF was then added, and the reaction was stirred for 1 hr at room temperature. Water (5 ml) was added, and the reaction was heated to 45°. Aqueous 10% sodium hydroxide (35 ml) and 35 ml of 30% hydrogen peroxide were added, and the reaction was further stirred for 1 hr at 45°. After this period, the reaction mixture was further diluted with water and extracted several times with methylene chloride. The organic extracts were dried ($MgSO_4$), filtered, and evaporated. The dark residue was purified by chromatography on 150 g of silica gel. Elution with 1:1 hexane–chloroform gave 6.7 g (65%) of the 2,4-dinitrophenylhydrazone alcohol **14**. Recrystallization from methanol gave the analytical sample: mp 196°; ir ($CHCl_3$) 3600, 3350, 1610, 1590, and 1500 cm^{-1}; nmr ($CDCl_3$) τ 0.90 (d, 1 H, J = 2 Hz), 1.72 (doublet of doublets, 1 H, J = 10 Hz, J' = 2 Hz), 2.10 (d, 1 H, J = 10 Hz), 8.60 (s, 3 H), and 9.00 and 9.20 (two doublets, 6 H, $J = J'$ = 7 Hz).

Anal. Calcd for $C_{20}H_{28}N_4O_5$: C, 59.39; H, 6.98. Found: C, 59.65; H, 7.11.

2α-Isopropyl-1β-hydroxy-4aβ-methyl-1,2,3,4,4a,7,8,8aβ-octahydronaphthalen-5(6H)-one (15).[16] The 2,4-DNP alcohol **14** (4.3 g, 0.0107 mol) was dissolved in 200 ml of ethyl acetate at −78° Ozone was passed through the solution *via* a fritted gas inlet tube at a rate of 2.3 g/hr for 1 hr (4 equiv). The solution was then concentrated to approximately 80 ml at the rotary evaporator. Methanol (300 ml) and 100 ml of saturated sodium bisulfite solution were added, and the solution was refluxed for 1 hr. The mixture was then cooled to room temperature, diluted with water, and extracted several times with ether. The ether extracts were washed with brine, dried ($MgSO_4$), filtered, and evaporated. The brownish oily residue was chromatographed on 50 g of alumina. Elution with ether gave 1.65 g (70%) of the desired keto alcohol **15** as a clear, colorless oil, homogeneous by vpc: ir (neat) 3450 (O–H) and 1705 cm^{-1} (C=O); nmr (CCl_4) τ 8.69 (s, 3 H) and 9.05 and 9.20 (two doublets, 6 H, J = 7 Hz).

(18) R. Greenwald, M. Chaykovsky, and E. J. Corey, *J. Org. Chem.*, **28**, 1128 (1963).

(19) According to the procedure of R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley & Sons, New York, N. Y., 1965, p 253.

A 2,4-DNP derivative of the product was made in the usual manner, mp 196°, undepressed on admixture with an authentic sample of **14**.

2α-Isopropyl-4aβ-methyl-1β-toluenesulfonyloxy-1,2,3,4,4a,7,8,-8aβ-octahydronaphthalen-5(6H)-one (16). The ketol **15** (460 mg, 2.04 mmol) was dissolved in 7 ml of dry pyridine under nitrogen. Toluenesulfonyl chloride (1.0 g, 5 mmol) was added and the solution let stand for 3 days at room temperature. Water (10 ml) was added to hydrolyze the excess tosyl chloride, and the solution was placed in the refrigerator for crystallization; 775 mg (100%) of the desired keto tosylate **16**, mp 145.5–146°, was collected.

3-Isopropyl-6-methyltricyclo[4.4.0.0^{2,8}]decan-7-one (17). A 0.50 *M* solution of dimethylsulfinyl carbanion in DMSO was prepared according to the procedure of Corey.[17] This solution (5 ml) was added to a solution of keto tosylate **16** (775 mg, 0.00204 mol) in 5 ml of DMSO under nitrogen, and the resulting solution was heated at 60° for 2 hr. After this period, the reaction was cooled, diluted with water, and extracted several times with ether. The ether extracts were washed with water and with brine, were dried ($MgSO_4$), filtered, and evaporated. The residue was distilled to yield 370 mg (90%) of the desired tricyclic product, homogeneous by vpc. An analytical sample was prepared by preparative vpc on a 10-ft 15% Carbowax 20M on Chromosorb W column: ir (neat) 1745 cm^{-1} (C=O); nmr (CCl_4) τ 9.01 (s, 3 H) and 9.02 (d, 6 H, J = 5 Hz).

Anal. Calcd for $C_{14}H_{22}O$: C, 81.49; H, 10.75. Found: C, 81.10; H, 10.80.

***dl*-Sativene (1).** The tricyclic ketone **17** (60 mg, 0.30 mmol) was dissolved in 5 ml of dry ether and added to 20 ml of a 5% solution of methyllithium in ether. The solution was refluxed for 2 days under nitrogen, then quenched with water. The ether layer was drawn off, and the aqueous layer was further extracted with ether. The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered, and evaporated. Ir showed complete absence of carbonyl absorption, and the presence of hydroxyl absorption at 3430 cm^{-1}. This crude alcohol was dissolved in 4 ml of dry pyridine under nitrogen at 0°. Thionyl chloride (0.1 ml) was added, and the reaction was stirred 30 min at 0°. Water was added and the mixture extracted several times with ether. The ether extracts were washed with cold 6 *N* hydrochloric acid and with saturated sodium bicarbonate, then were dried ($MgSO_4$), filtered, and evaporated. The clear oily residue was distilled to yield 50 mg of *dl*-sativene. The analytical sample was prepared by preparative vpc on a 10-ft 15% Carbowax 20M on Chromosorb W column: ir (CCl_4) 3060, 1660, and 885 cm^{-1}; nmr (CCl_4) τ 5.60 and 5.28 (singlets, 2 H), 8.95 (s, 3 H), 9.10 (d, 3 H, J = 5 Hz), and 9.13 (d 3 H, J = Hz); mass spectrum P$^+$ *m/e* 204.

Anal. Calcd for $C_{15}H_{24}$: C, 88.16; H, 11.84. Found: C, 87.88; H, 11.83.

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Isotope Effects on the Succinate Dehydrogenase–L-Chlorosuccinate System[1,2]

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Abstract: Relative rates, $rate_H/rate_D$, at pH 7.8, 30°, of succinate dehydrogenase, soluble and particulate-bound, catalyzed ferricyanide oxidation of several deuterated L-chlorosuccinates are, α-deuterio-, 1.1; β-*erythro*-deuterio-, 1.0; α,β-*threo*-dideuterio-, 2.1; β-*threo*-deuterio-, 2.0. A kinetic isotope effect is thereby established for breaking the β-*threo*-carbon–hydrogen bond of substrate and analysis of steady-state kinetic data obtained with normal substrate and α,β-*threo*-dideuterio-L-chlorosuccinate suggests that this isotope effect occurs at reduction of enzyme by substrate. Interpretation of steady-state data obtained with soluble enzyme is made on the basis of a previously proposed kinetic mechanism involving reoxidation of reduced enzyme before and after dissociation of product. Steady-state data obtained with particulate-bound enzyme require a mechanism involving reoxidation of reduced enzyme after dissociation of product and, in addition, competitive inhibition by ferricyanide. Experiments regarding application of an enol–hydride mechanism to the succinate dehydrogenase catalyzed oxidation of L-chlorosuccinate indicate little or no exchange prior to oxidation.

Succinate dehydrogenase (succinate: (acceptor) oxidoreductase, EC 1.3.99.1), a nonheme iron- and flavin-containing protein,[3] is of considerable interest with respect to its mechanism of action.[4] The enzyme catalyzes the dehydrogenation of L-chlorosuccinate[5,6] as well as the dehydrogenation of the natural substrate, succinate, and in both instances the unsaturated *trans* acid is obtained. Assuming the same *trans* arrangement of carboxyl groups for the reactive conformation of the substrates, then the hydrogens removed are also *trans*.[7,8] L-Chlorosuccinate thus possesses one set of oxidizable hydrogens while the natural substrate possesses two such sets.[7,9] L-Chlorosuccinate with one set of *trans*-oxidizable hydrogens is, then, particularly

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(2) A preliminary account of this work has appeared: O. Gawron, A. J. Glaid, III, K. Mahajan, G. Kananen, and M. Limetti, *Biochem. Biophys. Res. Commun.*, **25**, 518 (1966).

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