Microbiological Reduction of α,β-Unsaturated Ketones by *Beauveria sulfurescens*

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Microbiological reduction of α,β-unsaturated ketones was studied. Variously substituted cyclopentenones, cyclohexenones, and methylalkenones were reduced by *Beauveria sulfurescens* under low-aeration conditions. The reaction takes place only with a small substituent in the α-position and hydrogen in the β-position. The saturated ketone is always obtained, sometimes accompanied by saturated alcohol. Yields and optical purities of the products are excellent.

Much work has been published on the microbiological reduction of α,β-unsaturated ketones. Reduction of the double bond of 3-keto Δ^4 steroids has been carried out using *Clostridium paraputrificum*[1] or *Penicillium decumbens*.[2] However, in most cases reduction of the double bond was accompanied by formation of a hydroxyl group, e.g., when 3-keto Δ^4 steroids were treated with *Rhizopus nigricans*[3] or *Aspergillus niger*.[4] With cyclic α,β-unsaturated ketones, selective reduction of the double bond has seldom been observed,[5] the reaction generally leading to reduction of the ketone function as well. With acyclic α,β-unsaturated ketones, some examples of double bond reduction by yeasts have been reported,[5] but here again, alcohol formation was also regularly observed.

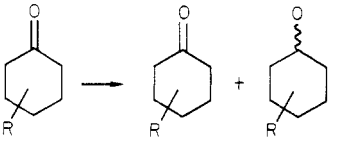
In the course of a study of microbial hydroxylation of cyclopentenone derivatives, we showed that some microorganisms (*Curvularia lunata, Aspergillus niger, Aspergillus ochraceus, Beauveria sulfurescens, Cunninghamella elegans*) known to be hydroxylating agents could reduce the double bond of α,β-unsaturated ketones when the reaction was carried out with low air flow. Following some encouraging preliminary results,[6] we undertook a study of the scope and limitations of this reduction reaction. The best results were obtained with *Beauveria sulfurescens* (ATCC 7159). Two series of substrates were studied: cycloalk-2-en-1-ones and acyclic α,β-unsaturated ketones.

(1) Reduction of Cycloalk-2-en-1-ones by *Beauveria sulfurescens*. In an earlier paper[6] we reported results concerning the reduction of a certain number of cyclopent-2-en-1-ones and cyclohex-2-en-1-ones. Further results for some cyclohex-2-en-1-ones are presented here (Table I).

The following conclusions may be drawn from these results. Reaction occurs if the substituent on the carbon of the double bond β to the carbonyl is hydrogen and if the substituent on the carbon of the double bond α to the carbonyl is not too bulky. When reaction leads to a compound containing an asymmetrical 2-carbon, this latter has the R configuration. Reduction of cyclopentenones gives exclusively cyclopentanones, while reduction of cyclohexenones yields a mixture of cyclohexanone and cyclohexanol.

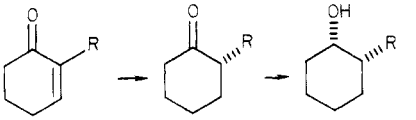
To check whether the cyclohexanols were formed after initial formation of the saturated ketone, we performed the following experiment. Cyclohexanone and 2-methylcyclohexanone were each placed in the incubation medium. In both cases, after 48 h, a mixture of saturated ketones and saturated alcohol was obtained, in proportions close to those found in the reduction of the corresponding unsaturated ketones, indicating that the reduction occurs according to eq 1.

Table I. Reduction of Cyclohex-2-en-1-ones by *Beauveria sulfurescens*



substituent	% yield: α,β-unsaturated ketone	saturated ketone	saturated alcohol
4-Me	0	65	35
5-Me (**1**)	0	70 (**2**)	30 (**3**)
6-Me (**4**)	0	30 (5[a])	70 (6[b])
2,6-DiMe	100	0	0
2,6,6-TriMe	100	0	0

[a] $[\alpha]^{25}_{578}$ + 6° (lit.[7] [α] +16.8°). [b] $[\alpha]^{25}_{578}$ +15° (lit.[8] $[\alpha]^{20}_{D}$ 24.3° (CH_3OH).



(1)

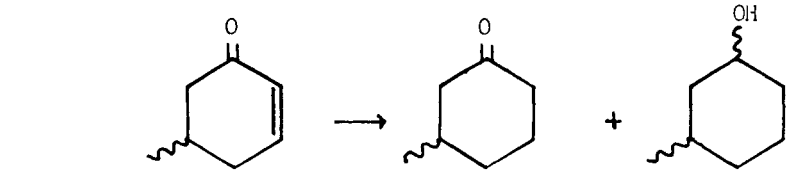
The *S* configuration of the 1-carbon of the cyclohexanols indicates attack on the carbonyl from the equatorial side, which is consistent with the results of Prelog[9] for numerous reductions of saturated ketones.

As shown by many authors,[10] whether the reaction is directed toward oxidation or reduction depends to a great extent of the pH of the reaction medium. At low pH, reduction of the ketone group is favored while at nearly neutral and high pH, oxidation of the alcohol group predominates. With the medium initially chosen for the present study (glucose, ammonium salt, and some mineral salts), the pH at the end of the incubation period was found to be about 2.5–3. Trials at various values of pH showed that the reduction of cyclohex-2-en-1-one no longer took place above pH 7.5. Thus, in order to favor the formation of the saturated ketone, the reaction was run at nearly neutral pH. Under these conditions, much better selectively could be obtained. When the culture medium

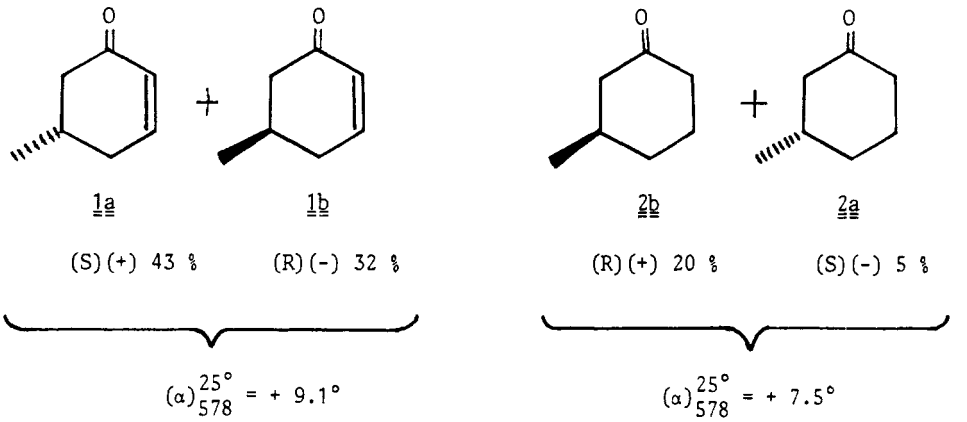
(1) A. Fauve and A. Kergomard, *Tetrahedron*, **37**, 899 (1981).
(2) T. L. Miller and E. J. Hessler, *Biochem. Biophys. Acta* **202**(2), 354 (1970).
(3) H. C. Murray and D. H. Peterson, U.S. Patent 2659743 (1953).
(4) H. C. Murray and D. H. Peterson, U.S. Patent 2649402 (1953).
(5) F. C. Fisher and O. Wiedemann, *Justus Liebigs Ann. Chem.*, **580**, 52 (1935).
(6) A. Kergomard, M. F. Renard, and H. Veschambre, *Tetrahedron Lett.*, 5197 (1978).
(7) J. Barry, A. Horeau, and H. Kagan, *Bull. Soc. Chim. Fr.* 989 (1970).
(8) B. Backstrom and B. Sjoberg, *Ark. Kemi.*, **26**, 549 (1967).
(9) V. Prelog, "3rd International Symposium of the IUPAC", Kyoto, Japan, 1964, Butterworths, London, 1964, p 119.
(10) I. Yamashita, K. Iino, and S. Yoshikawa, *Agric. Biol. Chem.*, **42**(6), 1125 (1978).

0022-3263/82/1947-0792$01.25/0

Scheme I



	1	2	3	cis	trans
48 h	0	70	30	78	22
24 h	0	74	26	55	45
9 h	0	90	10		
5 h	0	100	0		
2 h	75	25	0		



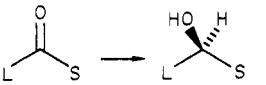
was buffered with calcium carbonate (6 g L^{-1}), the pH at the end of incubation was 5.5–6, and only cyclohexanone was obtained from cyclohex-2-en-1-one. When peptone was used instead of ammonium sulfate as a nitrogen source, the pH at the end of incubation was 5–5.5, and cyclohex-2-en-1-one yielded a mixture of 90% cyclohexanone and 10% cyclohexanol. Thus a change of pH makes possible selective reduction of the double bond.

Reduction of racemic 5-methyl- and 6-methylcyclohex-2-en-1-ones (Table I) was carried out to see whether any marked difference in rate could be detected between isomers. The double bond of (±)-5-methylcyclohex-2-en-1-one (**1**) was completely reduced after 5 h, giving (±)-3-methylcyclohexanone (**2**) as the only reaction product (see Scheme I). The conversion ratio was 25% after 2 h. When at this stage the saturated and unsaturated ketones were isolated, both were found to be optically active (as shown in Scheme I). The proportion of each enantiomer was calculated from the optical activity values of the pure compounds: (*R*)-(−)-5-methylcyclohex-2-en-1-one, $[\alpha]_D$ −90.17°;[11] (*R*)-(+)-3-methylcyclohexanone, (α) +12.56°.[11] Hence the rate constants for the reduction of each enantiomer (Table III). The 4-fold difference between the rate constants for **1a** and **1b** indicates a slight difference in activation energy.

The data in Scheme I show that reduction of the carbonyl group is much slower than that of the double bond. After 9 h of incubation, the proportion of saturated alcohol **3** was still only 10%. At this stage **3** was optically active, dextrorotatory, and mainly of trans configuration. A trans configuration corresponds to equatorial attack of hydrogen.

(11) N. L. Allinger and C. K. Riew, *J. Org. Chem.*, **40**, 1316 (1975).

The optical activity of **3** indicates that ketone **2a** (*S*) is reduced faster than ketone **2b** (*R*). This result would be expected from Prelog rules[9] (eq 2).

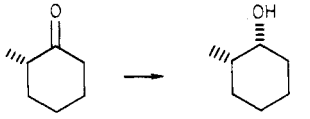


(2)

L = large substituent, S = small substituent

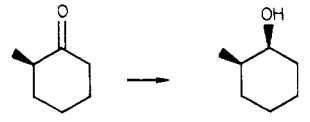
The trans alcohols so formed are subsequently isomerized to the thermodynamically more stable cis alcohols. The cis/trans ratio was 1/2 after 24 h and 3/5 after 48 h.

Racemic 6-methylcyclohex-2-en-1-one (**4**) after treatment with *B. sulfurescens* for 48 h yielded 30% saturated ketone **5** and 70% saturated alcohol **6** of cis configuration. Both products showed optical activity, resulting as above from preferential attack on one enantiomer of the saturated ketone **5** (eq 3a,b).



(3a)

(*S*)-(+)-**5a** (−)-(1*R*,2*S*)-**6a**

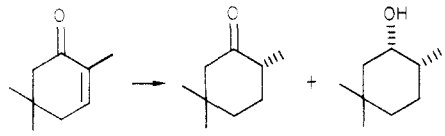


(3b)

(*R*)-(−)-**5b** (+)-(1*S*,2*R*)-**6b**

Here also, reduction of the carbonyl group was slower than that of the double bond of the unsaturated ketone,

Table II. Reduction of 2,5,5-Trimethylcyclohex-2-en-1-one by *Beauveria sulfurescens*



7 8 9

time	% yield 7	8	9
48 h	85	10	2–5
5 days	65	27	8
10 days	33	47	20

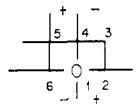
and the mixture of ketones **5a** and **5b** was dextrorotatory: $[\alpha]^{25}_{578}$ +6°, indicating a proportion of 80% of the *S* isomer **5a**. Similarly, the *cis*-2-methylcyclohexanol (**6**, $[\alpha]^{25}_{578}$ +15°) was calculated to contain 80% of the 1*S*,2*R* isomer **6b**. No cis–trans isomerization was observed, probably because of steric hindrance. As with the 3-methyl homologues, preferential attack from the equatorial side occurs, and isomer **5a** is reduced faster than **5b**.

Reduction of (±)-4-methylcyclohex-2-en-1-one yields mainly saturated ketone (65%) along with 35% of a mixture of cis and trans saturated alcohols, in which the trans isomer predominates.[9]

The reduction of homologous unsaturated ketones carrying bulkier substituents was also studied. 2,6-Dimethylcyclohex-2-en-1-one and 2,6,6-trimethylcyclohex-2-en-1-one were not reduced after 10 days of incubation.

2,5,5-Trimethylcyclohex-2-en-1-one (**7**) was reduced, though much more slowly than lesser substituted homologues (see Table II).

The optical rotations of both saturated ketone and alcohol are of opposite sign to that of their 2-methyl homologues. It may be assumed that the configuration of the asymmetrical carbon C-2 is *R* as in the 2-methyl homologue. The opposite sign can be explained by octant rules. As shown in the octant diagram given below, the *gem*-dimethyl group is located in a (+) octant.



octant diagram

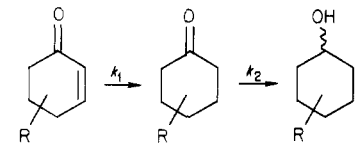
As would be expected from the diagram, the optical rotatory dispersion was positive. The saturated alcohol **9** derived from the saturated ketone consists only of the cis isomer in accordance with previous results; its configuration is 1*S*,2*R*.

No systematic study of reaction rates was made, but rate constants for the reduction of the double bond and of the carbonyl were determined in a few cases, assuming all reactions to be first order. Instances where two consecutive reactions occurred (A → B → C) were dealt with by using a classical treatment.[12] Results are given in Table III.

Rate constants of double bond reduction are close to 10^{-5} s^{-1}, and those for the carbonyl group are lower, about 10^{-6} s^{-1} for the cyclohexanones. They must be very much lower for the reduction of the carbonyl group of the cyclopentanones, given that no cyclopentanols were isolated,

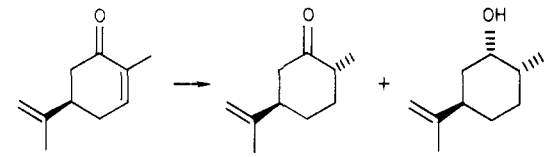
(12) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism", Wiley, New York 1961, p 166.

Table III. Rate Constants for the Reduction of Cyclohex-2-en-1-ones to Cyclohexanones and Cyclohexanols (20 °C)



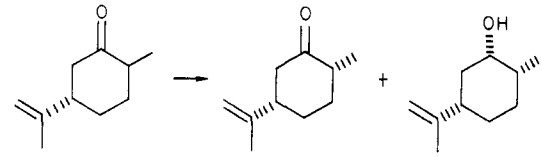
R	k_1, s^{-1}	k_2, s^{-1}
2-Me	0.8×10^{-5}	7.5×10^{-6}
(*S*)-5-Me	1.2×10^{-5}	2.5×10^{-6}
(*R*)-5-Me	5.4×10^{-5}	
2,5,5-Me	1×10^{-6}	1×10^{-6}

Table IV. Reduction of (+)-Carvone and (−)-Carvone[a]



10 11 12

	11	12
% yield	15	85
[α], deg	$[\alpha]^{25}_{578}$ +16.2	$[\alpha]^{25}_{578}$ +35
lit. [α], deg	$[\alpha]^{15}_{D}$ +13.9[14]	$[\alpha]_{D}$ +32.8[15]
name	(+)-dihydrocarvone	(+)-neodihydrocarveol



14 15

	14	15
% yield	40	60
[α], deg	$[\alpha]^{25}_{578}$ −38	$[\alpha]^{25}_{578}$ −28.6
lit. [α], deg	$[\alpha]_{D}$ −23.7[16]	$[\alpha]^{15}_{D}$ −28[16]
name	(−)-isodihydrocarvone	(−)-neoisodihydrocarveol

[a] Conditions: 48 h, aeration rate 10 mL/L/min.

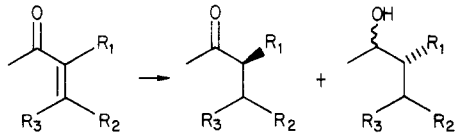
whereas cyclohexanols were isolated in all cases where reaction occurred.[13]

The reduction of two naturally occurring carvones was also studied to further investigate the stereochemistry of the reaction. Results for the reduction of (−)-carvone (**10**) and (+)-carvone (**13**) by *B. sulfurescens* are given in Table IV.

The reaction is evidently not slowed down by the presence of the 5-isopropenyl group. Reaction is complete after 48 h in both cases. The stereochemistry of the reduction products is consistent with the results obtained with the series of substituted cyclohex-2-en-1-ones described above. Thus, the saturated ketones **11** and **14** both have an asymmetrical 2-carbon with the same absolute configuration (*R*) as the other homologous cyclohexanones, and the corresponding saturated alcohols **12** and **15** are cis.

(13) T. A. Van Osselaer, G. L. Lemiere, J. A. Lepoire, and F. C. Alderweireldt, *Bull. Soc. Chim. Belg.*, **87**(2), 153 (1978).
(14) T. Nagasawa, *Osaka Kogzo Gijutsw Shikensho Hokoku*, **19**(4), 1 (1938).
(15) G. V. Pigulewsski, S. A. Kozhin, and V. G. Kosstenko, *Zh. Obshch. Khim.*, **28**, 1413 (1958).
(16) H. Schmidt, *Chem. Ber.*, **83**, 193 (1950).

Table V. Reduction of α,β-Unsaturated Methyl Ketones by *Beauveria sulfurescens*[a]



16 17 18

substituent[c]		% yield			$[\alpha]^{25}_{578}$ for 17, deg
R_1	R_2	16	17	18	
n-Bu	Me	0	95[b]	0	
Me	Et	0	90	5	+15
Me	*n*-Pr	0	92	3	+11
Me	*n*-Bu	100	0	0	
Me	*n*-Pent	100	0	0	

[a] Conditions: 48 h, aeration rate 10 mL/L/min. [b] Results for 96-h reaction time. [c] R_3 = H.

Optical purity of the products obtained is high (compared with reported values).

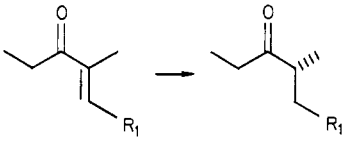
If these results are compared with those obtained by Noma et al.[17] for the reduction of the same two carvones with *Pseudomonas ovalis*, it is clear that reduction with *B. sulfurescens* is considerably more stereoselective. Thus with (+)-carbone Noma obtained both isomers of dihydrocarvone together with all four isomers of dihydrocarveol (isodihydrocarvone, isodihydrocarveol, and neoisodihydrocarveol, accounting for 80% of the reaction products).

(2) Reduction of Acyclic α,β-Unsaturated Ketones by *Beauveria sulfurescens*. Previous results[6] concerning reduction of α,β-unsaturated methyl ketones, and those summarized in Table V, lead to the following conclusions.

When reaction does take place, the corresponding saturated ketones are mainly obtained, along with small amounts of saturated alcohol. This amount is significant only with shorter chain ketones. However, the size of the α substituent did not have a large effect on reaction rate. With an ethyl group α to the carbonyl on the double bond, reaction was complete after 48 h, while with an *n*-butyl group in this position complete reaction occurred in 96 h. As with the cycloalk-2-en-1-ones, the presence of a hydrogen atom β to the carbonyl is necessary for reaction to occur. Furthermore, the size of the β-substituent R_2 seems critical, since no reaction occurred when R_2 = *n*-Bu or *n*-Pent even though reaction was complete when R_2 = *n*-Pr. Whenever reaction did occur, excellent yields were obtained, and products were optically pure. The configuration of the asymmetrical carbon α to the carbonyl is *S* when R_1 = methyl whereas in the corresponding cycloalkanone series it is *R*. An explanation of this inversion of configuration is proposed below (Stereochemical Course of the Reaction).

Data for 3-methylhexan-2-one and 3-methylheptan-2-one were lacking, but homologous compounds with the same absolute configuration are described in the literature and have the same sign of optical rotation. Optical purity was checked by ^{1}H NMR using a europium chiral shift reagent. When R_1 = methyl, the methyl signal in a racemate appears as two doublets, one for each enantiomer. In compounds obtained here only one doublet was observed, indicating an optical purity of more than 95%.

Table VI. Reduction of α,β-Unsaturated Ethyl Ketones by *Beauveria sulfurescens*[a]



19 20

	% yield		
R_1	19	20	$[\alpha]^{25}_{578}$, deg (lit.)
CH_3	0	90	−29 (+30)[18]
C_2H_5	0	90	−23 (−21.5)[19]

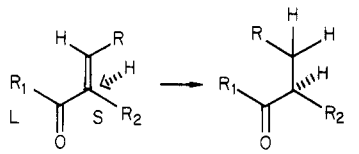
[a] Conditions: 48 h, aeration rate 10 mL/L/min.

Reduction of α,β-unsaturated ethyl ketones of type **19** was also studied. The results obtained are summarized in Table VI.

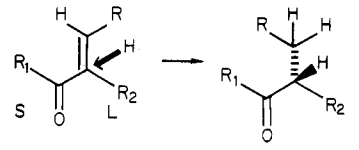
Ketones of type **19** were completely reduced in 48 h, giving exclusively the corresponding saturated ketones. The asymmetrical 4-carbon created in saturated ketones **20** has the *R* configuration. This configuration is the opposite of that obtained in reduction products of α,β-unsaturated methyl ketones (see Table V). An explanation of this result is proposed below.

(3) Stereochemical Course of the Reaction. It is established that addition of hydrogen is trans.[20,21] The asymmetrical carbon α to the carbonyl in the reduction product has an *R* configuration in the cycloalkanones, whether the α substituent is Me or D. 3-Deuteriocycloalk-2-en-1-ones are reduced, giving 3*S* saturated cyclic ketones.[20] We assume here that addition is trans in acyclic ketones, though this is as yet unconfirmed.

A general explanation is possible which accounts for the absolute configurations of the products obtained. The two double bond substituents α to the carbonyl are classified in a way analogous to that used in the Prelog rule.[9] Attack of hydrogen occurs at this carbon according to the same stereochemistry as at the carbonyl carbon, i.e., from behind if the larger substituent is on the left and from the front if the larger substituent is on the right (eq 4a,b).



(4a)



(4b)

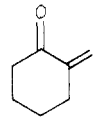
This rule accounts for the inverse stereochemistry for methyl ketones on the one hand and ethyl ketones and cyclic ketones on the other. For methyl ketones **16** (Table V) the methyl group of 3-methylpent-3-en-2-one (**16**; R_1 = R_2 = CH_3, R_3 = H) R_1 is larger than the acetyl group. The situation is different for the ethyl ketone **19** (R_1 = CH_3 or C_2H_5, Table VI) in which the methyl group is smaller than the propionyl group.

(17) (a) Y. Noma and C. Tatsumi, *Nippon Noukagoka Kaishi*, 47, 705 (1953); (b) Y. Noma, S. Nonomura, H. Veda, and C. Tatsumi, *Argic. Biol. Chem.*, 38, 735 (1974).
(18) D. Enders and H. Eichenauer, *Angew. Chem.*, 91(5), 425 (1979).
(19) R. G. Riley and R. M. Silverstein, *Tetrahedron*, 30, 1171 (1974).
(20) G. Dauphin, J. C. Gramain, A. Kergomard, M. F. Renard, and H. Veschambre, *Chem. Commun.* 318 (1980).
(21) G. Dauphin, J. C. Gramain, A. Kergomard, M. F. Renard, and H. Veschambre, *Tetrahedron Lett.*, 4275 (1980).

It is also noteworthy that the 3-carbon of the saturated acyclic alcohols **18** does not have the same configuration as the 3-carbon of the corresponding saturated ketones. It seems that in the acyclic series, formation of the alcohol is not by reduction of the saturated ketone, as was shown to be the case in the cyclic series. Indeed, 2-pentanone and 3-methylpentan-2-one were found to be unaffected by 60 h of incubation with *B. sulfurescens*. Given that the mode of formation of the saturated acyclic alcohol is different from that of their cyclic analogues, it would appear that in this case, the saturated alcohol arises from reduction of the α,β-unsaturated alcohol. It has been shown that acyclic α,β-unsaturated alcohols are reduced by *B. sulfurescens*.[22] It may therefore be assumed that the group CH_3CHOH is larger than the methyl group.

For the 2-methylcyclohex-2-en-1-ones, it may be assumed that the methyl group is smaller than the ring segment attached to the 2-carbon. This rule is consistent with two other experimental results.

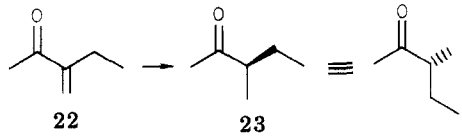
2-Methylenecyclohexanone (**21**) after 48 h of incubation



21

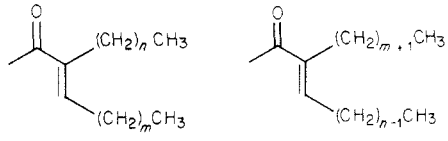
with *B sulfurescens*, yielded a mixture of 2-methylcyclohexanone (50%) and 2-methylcyclohexanol (50%). The two reaction products were found to have the same absolute configuration as those obtained from 2-methylcyclohex-2-en-1-one (2*R* for the saturated ketone, 1*S*,2*R* for the saturated alcohol). In this case, of the two halves of the ring, that which contains the α-carbonyl group is the smaller.

Similarly, 3-methylenepentan-2-one (**22**) yielded almost exclusively the saturated ketone **23** (eq 5) with $[\alpha]^{25}_{578}$ −21°, i.e., (*R*)-(−)-3-methylpentan-2-one. As already mentioned,[6] 3-methylpent-3-en-2-one gave the saturated ketone (*S*) with $[\alpha]^{25}_{578}$ +22.5°.



22 23 (5)

This example suggests that more generally it is possible to obtain each of the two enantiomers of an α-substituted ketone by microbiological reduction of ketones **24** and **25**.



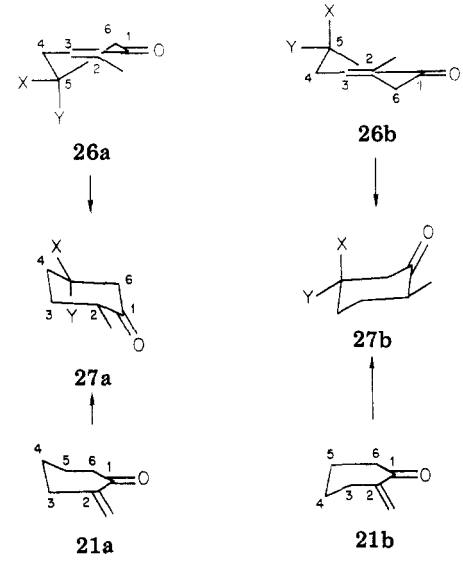
24 25

This rule is also applicable to the reduction of α,β-unsaturated aldehydes by *B. sulfurescens*.[22] Here, the CHO group is smaller than the methyl group.

A further conformational factor may be involved in the case of cycloalkenones.

2-Methylcyclohex-2-en-1-one (X = Y = H) can exist in the two enantiomeric conformations **26a** and **26b** (see Scheme II). In both conformations, carbons 1–4 are in the same plane, the 6-carbon is quite close to this plane,

Scheme II



and the 5-carbon well outside it, to one side or the other. If we now assume that the enantiomer which reacts more readily (with chiral enzyme) is that having the helicoidal shape 4–5–6–1 nearest to that of the reduction product, according to the principle of least movement, then according to this principle **26a** should give **27a** faster than **26b** should give **27b**.

The same reasoning may be applied to the reduction of 2-methylenecyclohexanone (**21**). Here there is no plane containing four carbons, but the 4-carbon is situated furthest away from the plane containing carbons 1–3. Isomer **21a**, in which the 4-carbon is above the plane, will give **27a** with conservation of the helicoidal shape of carbons 3–6 faster than **21b** should give **27b**.

The above reasoning can also explain why (*R*)-(−)-5-methylcyclohex-2-en-1-one reacts faster than its (*S*)-(+) isomer. The most stable conformation of the (*R*)-(−) isomer is probably **26a** (X = CH_3, Y = H), while that of the (*S*)-(+) isomer is probably **26b** (X = H, Y = CH_3). Thus if, in general, **26a**-type isomers react more quickly than **26b**-type isomers to give **27a**-type products, it would be expected that the *R* enantiomer should react faster than the *S* enantiomer.

Conclusion

Provided certain conditions are fulfilled (mainly the presence of hyrogen β to the carbonyl), *B. sulfurescens* reduces a wide variety of α,β-unsaturated ketones to give the corresponding saturated ketone along with a small proportion of the corresponding saturated alcohol, the amount of which depends[5] on the structure of the starting material. The reaction is highly stereoselective. In those cases where an asymmetrical carbon is formed, the optical purity of the product is close to 100%.

In previous work[20,21] it had been shown that the microbiological reduction is trans in the case of cyclic ketones. It may be assumed that the same stereochemistry occurs for acyclic ones.

The scope of this useful biological reducing agent had already been studied for α,β-unsaturated aldehydes[22] and is being further investigated by using other α,β-unsaturated carbonyl compounds as substrates.

Experimental Section

Optical rotations were measured with a Perkin-Elmer 141 polarimeter at the yellow mercury J line (λ = 578 nm) at 25 °C. ^{1}H NMR spectra were obtained by using Perkin-Elmer R24 and

(22) M. Desrut, A. Kergomard, M. F. Renard, and H. Veschambre, *Tetrahedron 37*, 3825 (1981).

JEOL CX 60 instruments for $CDCl_3$ solutions. Chemical shifts are given relative to Me_4Si as an internal standard. Column chromatography was performed on 70–230-mesh Merck silica gel with pentane/ether as the mobile phase. Gas chromatography was performed by using an Intersmat IGC 12 M chromatograph equipped with a catharometer. Columns were 10 ft × 0.125 in. stainless steel packed with 20% Carbowax 20M on Chromosorb W. Hydrogen was used as the carrier gas.

General Methods. The microorganism *Beauveria sulfurescens* (ATCC 7159) was grown for 24 h at 27 °C with an aeration rate of 10 mL L^{-1} min^{-1} in the following culture medium: glucose, 30 g L^{-1}; $(NH_4)_2SO_4$, 2 g L^{-1}; K_2HPO_4, 1 g L^{-1}; $MgSO_4$, 0.5 g L^{-1}; KCl, 0.5 g L^{-1}; $ZnSO_4$, 0.3 g L^{-1}; $FeSO_4 \cdot 7H_2O$, 0.01 g L^{-1} in tap water. The culture was grown in a Biolafitte 2-L fermentation tank equipped with a mechanical stirrer, sterile air inlet, thermostat, and pH probe. After the initial 24-h period, 600–700 mg L^{-1} of substrate was added as a solution in 2–3 mL of Me_2SO. After 48 h at 20 °C with the same aeration rate, the contents of the tank were filtered, saturated with ammonium sulfate, and extracted four times with ether. A large amount of emulsion was always formed during extraction. When clean phase separation could not be achieved even after 24 h of settling, centrifuging at 3000 rpm for 30 min was necessary. The ether extracts were evaporated to dryness on a water bath at atmospheric pressure, leaving a brown oil which was then analyzed by gas chromatography. Yields were determined by using an internal standard. The crude products were purified by column chromatography with Merck 60 silica gel and pentane/ether (90/10) as the eluant.

4-Methylcyclohex-2-en-1-one. This was prepared from 4-methylanisol according to Dauben et al.[23] The products obtained after purification were 4-methylcyclohexanone (65%) and 4-methylcyclohexanol (35%). The latter product proved to be a mixture of cis (15%) and trans (85%) isomers by ^1H NMR. Their structure was confirmed by comparison of their NMR spectra with those of authentic samples.

5-Methylcyclohex-2-en-1-one was prepared from ethyl crotonate and ethyl acetoacetate according to Blanchard et al.[24] After 9 h of incubation the two products formed were purified and their structures confirmed by comparison of their NMR spectra with those of authentic samples. For 3-methylcyclohexanol (**3**), $[\alpha]^{25}_{578}$ +5° (*c* 0.06, $CHCl_3$) (lit.[25] $[\alpha]^{21}_{D}$ +6.7°). After a reaction time for 2 h, the two products formed were isolated: 5-methylcyclohex-2-en-1-one (**1**), $[\alpha]^{25}_{578}$ +9.1° (*c* 0.19, $CHCl_3$) (lit.[11] $[\alpha]^{25}_{D}$ −90.17° (*c* 0.767, $CHCl_3$)); 3-methylcyclohexanone (**2**), $[\alpha]^{25}_{578}$ +7.5° (*c* 0.03, $CHCl_3$) (lit.[11] $[\alpha]^{25}_{D}$ +12.56° (neat)).

6-Methylcyclohex-2-en-1-one was prepared by Birch reduction of *o*-toluidine according to Dauben et al.[23] Two products having the same retention times as 2-methylcyclohexanone (30%) and 2-methylcyclohexanol (70%) were located. After purification their structure was confirmed by comparison of their NMR spectra with those of authentic samples: 2-methylcyclohexanone, $[\alpha]^{25}_{578}$ +6° (*c* 0.09, $CHCl_3$) (lit.[7] $[\alpha]$ +16.8°); 2-methylcyclohexanol, $[\alpha]^{25}_{578}$ +15° (*c* 0.06, $CHCl_3$) (lit.[8] $[\alpha]^{20}_{D}$ +24.3° (CH_3OH)).

2,5,5-Trimethylcyclohex-2-en-1-one (7) was prepared from methyldimedon[26] according to Ellis et al.[27] The crude product obtained after 10 days incubation was purified, giving starting material (33%), saturated ketone (47%), and saturated alcohol (20%). The structure of the saturated ketone was confirmed by comparison of its NMR spectrum with that of an authentic sample. The optical rotary dispersion of (*R*)-(+)-2,5,5-trimethylcyclohexanone (**8**) was as follows [λ nm ($[\alpha]^{25}$ ($CHCl_3$), deg)]: 578 (+8), 546 (+8.5), 436 (+24.5).

The structure of *cis*-(1*S*,2*R*)-2,5,5-trimethylcyclohexanol (**9**) was confirmed by comparison of its NMR spectrum with that of *cis*-2-methylcyclohexanol; $[\alpha]^{25}_{578}$ −11° (*c* 0.027, $CHCl_3$).

(−)-Carvone (10) was a commercial product. After purification we obtained two products: (+)-dihydrocarvone (**11**, 15%) and (+)-neodihydrocarveol (**12**, 85%). Their structures were confirmed by comparison of their NMR spectra with those obtained by NOMA[17a] and for (+)-neodihydrocarveol with that of *cis*-2-methylcyclohexanol: (+)-dihydrocarvone (**11**), $[\alpha]^{25}_{578}$ +16.2° (*c* 0.054 $CHCl_3$) (lit.[4] $[\alpha]^{15}_{D}$ +13.9°); (+)-neodihydrocarveol (**12**), $[\alpha]^{25}_{578}$ +35° (*c* 0.204, $CHCl_3$) (lit.[15] $[\alpha]_{D}$ +28°).

(+)-Carvone (13) was a commercial product. After separation we obtained two products: (−)-isodihydrocarvone (**14**, 40%) and (−)-neoisodihydrocarveol (**15**, 60%). Their structures were confirmed by comparison of their NMR spectra with those obtained by NOMA et al.:[17b] (−)-isodihydrocarvone (**14**), $[\alpha]^{25}_{578}$ −38° (*c* 0.062, $CHCl_3$) (lit.[16] $[\alpha]_{D}$ −23.7°); (−)-neoisodihydrocarveol (**15**), $[\alpha]^{25}_{578}$ −28.6° (*c* 0.051, $CHCl_3$) (lit. $[\alpha]^{15}_{D}$ −28°).

2-Methylenecyclohexanone (21) was prepared from the α-methylene ketal of cyclohexanone[28] according to Huet et al.[29] The 2-methylcyclohexanone (50%) and 2-methylcyclohexanol (50%) were separated: 2-methylcyclohexanone, $[\alpha]^{25}_{578}$ −15° (*c* 0.02, $CHCl_3$) (lit.[7] $[\alpha]$ +16.8°); 2-methylcyclohexanol, $[\alpha]^{25}_{578}$ +22.1° (*c* 0.04, $CHCl_3$) (lit.[8] $[\alpha]$ +24.3°).

Cyclic Ketones Not Reduced by *B. sulfurescens*. 2,6-Dimethylcyclohex-2-en-1-one prepared from 2,6-dimethylcyclohexanone according to Trost et al.[30] and 2,6,6-trimethylcyclohex-2-en-1-one prepared from 2,6,6-trimethylcyclohexanone[31] according to Meinwald et al.[32] were recovered unchanged even after a 10-day reaction time.

3-Butylpent-3-en-2-one was prepared from heptan-2-one and acetaldehyde according to Levy et al.[33] After a 48-h reaction time, analysis of the crude product showed the presence of 50% 3-butylpent-3-en-2-one and 50% 3-ethylheptan-2-one. After 96 h of incubation, however, the crude product contained only 3-ethylheptan-2-one (structure confirmed by NMR).

3-Methylhex-3-en-2-one was prepared from methyl ethyl ketone and propionaldehyde according to Levy et al.[33] Two products were formed and separated. One was (*S*)-(+)-3-methylhexan-2-one: 90% yield; structure confirmed by NMR; $[\alpha]^{25}_{578}$ +15° (*c* 0.04, $CHCl_3$). The optical purity (>95%) was determined by comparison of NMR spectra of racemic and optically active product in the presence of tris[3-[(trifluoromethyl)hydroxymethylene]-*d*-camphorato]europium(III). The second product was 3-methylhexan-2-ol (5% yield) which was identified by its GC retention time compared to that of an authentic sample.

3-Methylhept-3-en-2-one was prepared from butanone and butyraldehyde according to Levy et al.[33] Two products were formed and separated. One was (*S*)-(+)-3-methylheptan-2-one: 92% yield; structure confirmed by NMR; $[\alpha]^{25}_{578}$ +11° (*c* 0.1, $CHCl_3$). The optical purity (>95% yield) was determined as for (*S*)-(+)-3-methylhexane-2-one. The second product was 3-methylheptan-2-ol (3% yield), which was identified only by comparison of its GC retention time with that of an authentic sample.

3-Methylenepentan-2-one was prepared from methyl propyl ketone and formaldehyde according to Dubois.[34] Two products were formed and separated (*S*)-(−)-3-methylpentan-2-one: 65% yield; structure confirmed by NMR; $[\alpha]^{25}_{578}$ −21° (*c* 0.1, $CHCl_3$) (lit.[35] for (3*R*)-(+)-3-methylpentan-2-one $[\alpha]$ +24.9°). (3*R*)-(+)-3-Methylpentan-2-ol: 10% yield; structure confirmed by comparison of its NMR spectrum with that of an authentic sample; $[\alpha]^{25}_{578}$ +11° (*c* 0.108, $CHCl_3$) (lit.[36] for (3*S*)-(−)-3-methylpentan-2-ol $[\alpha]$ −11.5°).

Acyclic α,β-Unsaturated Ketones Not Reduced by *B. sulfurescens*. 4-Methylpent-3-en-2-one was a commercial

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(28) F. Huet, A. Lechevallier, and J. M. Conia, *Synth. Commun.*, **10**, 83 (1980).
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(30) B. M. Trost, R. N. Salzmann, and K. Hiroi, *J. Am. Chem. Soc.*, **98** 4887 (1976).
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(32) J. Meinwald and C. C. Cornwall, *J. Am. Chem. Soc.*, **77**, 5991 (1955).
(33) J. Levy and R. M. Lusskin, U.S. Patent 2 889 339 (1959).
(34) J. E. Dubois, *Ann. Chim. (Paris)*, **12**(6), 406 (1951).
(35) C. Djerassi and L. E. Geller, *J. Am. Chem. Soc.*, **81**, 2789 (1959).
(36) R. Rossi, P. Pino, P. Piacenti, L. Lardici, and G. Delbino, *Gazz. Chim. Ital.*, **97**(8), 1194 (1967).

product. 3,4-Dimethylpent-3-en-2-one was prepared from 2-methyl-2-butene and acetylchloride according to House et al.[37] 3-Methyloct-3-en-2-one was prepared from methyl ethyl ketone and pentaldehyde according to Levy et al.[33] 3-Methylnon-3-en-2-one was prepared from methyl ethyl ketone and *n*-heptaldehyde according to Levy et al.[33]

All these products were recovered unchanged even after 5 days of incubation.

4-Methylhex-4-en-3-one was prepared from diethyl ketone and acetaldehyde according to Levy et al.[33] One product formed and was purified. (*R*)-(−)-4-Methylhexan-3-one: 95% yield; structure confirmed by NMR; $[\alpha]^{25}_{578}$ −29° (*c* 0.118, $CHCl_3$) (lit.[18] $[\alpha]$ −30°). The optical purity (>95%) was confirmed by comparison of NMR spectra of racemic and optically active product in the presence of tris[3-[(trifluoromethyl)hydroxymethylene]-*d*-camphorato]europium(III).

4-Methylhept-4-en-3-one was prepared from diethyl ketone and propionaldehyde according to Levy et al.[33] One product formed and was purified. (*R*)-(−)-4-Methylheptan-3-one: 95% yield; structure confirmed by NMR; $[\alpha]^{25}_{578}$ −23° (*c* 0.12, $CHCl_3$) (lit.[19] $[\alpha]^{27}_{D}$ −21.5° (*c* 1, hexane).

Experiments Using Culture Medium with Modified pH. Method a. Solid Ca CO_3 (6 g L^{-1}) was added to the standard medium described above. Under these conditions cyclohex-2-en-1-one in the usual concentration gave only cyclohexanone.

Method b. Ammonium sulfate was replaced by 10 g L^{-1} of peptone in the same standard culture medium. Under these conditions cyclohex-2-en-1-one in the usual concentrations gave cyclohexanone (90%) and cyclohexanol (10%).

Acknowledgment. We thank Dr. F. Huet (Orsay) for a sample of the 2-methylene ketal of cyclohexanone and Dr. G. Dauphin and J. C. Gramain (Clermont) for stimulating discussions bearing on this study.

Registry No. (*S*)-(+)-**1a**, 15466-88-3; (*R*)-(−)-**1b**, 54307-74-3; (*S*)-(−)-**2a**, 24965-87-5; (*R*)-(+)-**2b**, 13368-65-5; (±)-*cis*-**3**, 24965-90-0; (±)-*trans*-**3**, 23068-71-5; (±)-**4**, 67120-83-6; (*S*)-(+)-**5a**, 22554-27-4; (*R*)-(−)-**5b**, 22554-29-6; (−)-(1*R*-2*S*)-**6a**, 19043-02-8; (+)-(1*S*,2*R*)-**6b**, 15963-35-6; **7**, 42747-41-1; (*R*)-(+)-**8**, 79918-73-3; (1*S*,2*R*)-**9**, 79918-74-4; (−)-**10**, 6485-40-1; (+)-**11**, 5524-05-0; (+)-**12**, 20549-48-8; (+)-**13**, 619-02-3; (−)-**14**, 53796-79-5; (−)-**15**, 53796-80-8; **16** (R_1 = Bu; R_2 = Me), 79918-75-5; **16** (R_1 = Me; R_2 = Et), 1187-80-0; **16** (R_1 = Me; R_2 = Pr), 39899-08-6; **16** (R_1 = Me; R_2 = Bu), 60438-53-1; **16** (R_1 = Me; R_2 = Pent), 54615-56-4; (*R*)-**17** (R_1 = Bu; R_2 = Me), 69856-95-7; (*S*)-**17** (R_1 = Me; R_2 = Et), 79980-77-1; (*S*)-**17** (R_1 = Me; R_2 = Pr), 69856-94-6; **18** (R_1 = Me; R_2 = Et), 2313-65-7; **18** (R_1 = Me; R_2 = Pr), 31367-46-1; **19** (R = Me), 52883-78-0; **19** (R = Et), 22319-31-9; (*R*)-**20** (R = Me), 77858-08-3; (*R*)-**20** (R = Et), 51532-31-1; **21**, 3045-98-5; (±)-4-methylcyclohex-2-en-1-one, 79980-78-2; 4-methylcyclohexanone, 589-92-4; *cis*-4-methylcyclohexanol, 7731-28-4; *trans*-4-methylcyclohexanol, 7731-29-5; 3-methylenepentan-2-one, 4359-77-7; (*S*)-(−)-3-methylpentan-2-one, 2695-53-6; 3-methylpentan-2-ol, 365-60-6; 4-methylpent-3-en-2-one, 141-79-7; 3,4-dimethylpent-3-en-2-one, 684-94-6; (±)-5-methylcyclohex-2-en-1-one, 54352-35-1.

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Synthesis of Large-Ring Analogues of Estrone by a Ring-Expansion Route

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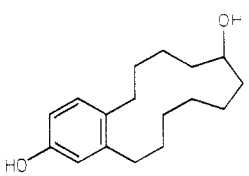
Received August 25, 1981

A synthetic methodology is described wherein a sequence of three ring expansions is used to convert cycloheptanone to 4′-methoxy-5,6-benzocyclodecenone, which was tested for estrogenic properties but showed no uterotrophic activity. Attempts to selectively expand the large ring by one more carbon to 8,9:13,14-diseco-18-norestrone were not successful.

We recently reported[1] the synthesis of 8,9:13,14-diseco-18-norestradiol, **1**, which is the first analogue of the human sex hormones wherein the B, C, and D rings are replaced by a single ring. The present paper describes an alternative synthetic route directed toward compound **2**, which is another member of this general class of large-ring hormone analogues. The preparation of these compounds is part of a program to determine to what extent these flexible analogues will mimic the biological properties of the corresponding steroidal hormones which are quite rigid.[2]



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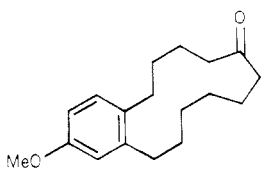
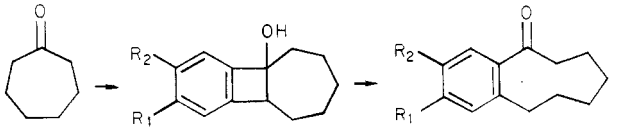


2

(1) Thies, R. W.; Yue, S. *J. Chem. Soc., Chem. Comm.* **1980**, 950.
(2) Biological activity has been observed in several mono seco cases; e.g., Voight, W.; Castro, A.; Covey, D. F.; Robinson, C. H. *Acta Endocrinol.* **1978**, *87* 668 and earlier papers in that series; Morrow, D. F.; Dallow, D. *Ann. Rep. Med. Chem.* **1971**, *7*, 182; Crossley, N. S., *J. Chem. Soc. C* **1971**, 2491.

Results and Discussion

Synthesis of compound **2** requires that a substituted benzo unit be fused to a large ring in a particular position relative to the carbonyl group. Relatively few methods have been reported for attaching benzo moieties to medium or large rings and still fewer for substituted benzo cases.[3] This synthesis utilizes a variation of a reaction developed by Caubere,[4] which simultaneously expands a ring ketone and attaches the benzo unit; e.g., cycloheptanone had been converted to the cyclobutanol **3a** which can then be rearranged to the benzocyclononanone **4a**. In the present



3a, R_1 = H; R_2 = H
b, R_1 = OMe; R_2 = H
c, R_1 = H; R_2 = OMe

4a-c

case, cycloheptanone was treated with sodium amide and

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