values[6] reported for such sterols. This must have been due to a structural change close to the alcohol function, since the stereochemistry of the ring junctures was clearly of the usual steroid type as demonstrated by rotatory dispersion measurements[7] of the ketones II, IV, V, VI as well as of appropriate Δ^{8}-7-ketones, $\Delta^{8(14)}$-7-ketones and Δ^{14}-16-ketones of the lophenol series, which were all very similar[8] to the dispersion curves of corresponding ketones in the cholestane or ergostane series.

An important clue to this structural change was provided by three observations: (a) None of the above analytical data excluded a C_{27} or C_{29} formula and hence lophane (III) was submitted to mass-spectrographic molecular weight determination[9] which proved a $C_{28}H_{50}$ formula (Calcd. 386.7; found: 386.3 ± 0.6). (b) While the molecular weight determination implied an ergostane side chain (C_9), pyrolysis and vapor phase chromatography of the volatile fragments[10] clearly showed that lophenol possessed a C_8 side chain of the cholestane type, which suggested that an extra methyl group had to be present in the ring. The above described chemical transformations (see formation of VI as well as reformation of I from lophenone with $LiAlH_4$) had already excluded C-5, C-9 and C-14 as possible points of substitution and the abnormal molecular rotation difference calculations suggested proximity to ring A (the presence of the alcohol function at C-3 being assumed by the shape of the rotatory dispersion curve[7] of derived ketones). (c) Exhaustive brominations[11] of lophanone (II) led to ambiguous results, apparently due to spontaneous dehydrobromination and consequent excessive up-take of reagent, but the fact that the rotatory dispersion curve (methanol solution) was unchanged in the presence of hydrochloric acid (in contrast to cholestan-3-one where hemiketal formation is observed) indicated alkyl substitution α to the ketone function (unpublished experiments by L. A. Mitscher). Using the rotatory dispersion curve of II as evidence for the location of the carbonyl group at C-3, these results require the location of a methyl group at C-2 or at C-4. Indeed, lophanone (II) was found by direct comparison (mixture melting point determination, infrared and rotatory dispersion comparison) to be identical with synthetic[12] 4α-methylcholestan-3-one from which it follows that lophenol (I) is 4α-methyl-Δ^{7}-cholesten-3β-ol.

The presence of a cholestane derivative in a plant is noteworthy. Even more striking is the existence of a 4-methylsterol in nature which together with the recently announced structure of cycloeucalenol[13] —a 4-monomethyl triterpenoid—strongly points toward demethylation of squalene cyclization products as a biosynthetic route to plant sterols. Such a course has so far been demonstrated by direct biochemical experimentation only in animals.[14,15]

(6) D. H. R. Barton and W. Klyne, *Chemistry and Industry*, 755 (1948).

(7) For references see C. Djerassi, *Bull. soc. chim. France*, 741 (1957).

(8) The preparation of these ketones and the rotatory dispersion results will be reported in a detailed paper.

(9) P. de Mayo and R. I. Reed, *Chemistry and Industry*, 1481 (1956); we are grateful to Dr. de Mayo for this determination.

(10) This valuable technique for the determination of steroid and terpene side chains has been developed by Prof. E. R. H. Jones and collaborators at Oxford University. We are greatly indebted to them for advance information on this unpublished method and to Dr. L. B. High of Oxford University making out the side chain determination.

(11) This technique has been used successfully in the triterpene series by C. S. Barnes, D. H. R. Barton, A. R. H. Cole, J. S. Fawcett and B. R. Thomas, *J. Chem. Soc.*, 571 (1953).

(12) G. D. Meakins and O. R. Rodig, *ibid.*, 4679 (1956); J. L. Beton, T. G. Halsall, E. R. H. Jones and P. C. Phillips, *ibid.*, 753 (1957). We are indebted to these investigators for a gift of 4α-methylcholestan-3-one.

(13) J. S. G. Cox, F. E. King and T. J. King, *Proc. Chem. Soc.*, 290 (1957).

(14) See F. Gautschi and K. Bloch, THIS JOURNAL, **79**, 684 (1957).

(15) NOTE ADDED IN PROOF.—W. W. Wells and D. H. Neiderhiser, THIS JOURNAL, **79**, 6569 report a sterol from rat feces which is believed to be 4α-methyl-Δ^{7}-cholesten-3β-ol. A mixture melting point of lophenol and of its acetate with that of the sterol and its acetate kindly supplied by Dr. Wells, gave a depression of 4° and 10°, respectively. A small difference in the infrared spectra of the two sterols was observed.

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RACEMIZATION OF LYSINE BY PROTEUS VULGARIS

Sir:

We have discovered that *Proteus vulgaris* ATCC 4669 contains a highly active catalytic system for racemizing lysine. The conversion of L-lysine to DL-lysine by an autolyzed cell suspension of this organism[1] is shown by the data in Table I.

TABLE I

RACEMIZATION OF L-LYSINE BY AN AUTOLYSATE OF *Proteus vulgaris*

Reaction system[a]	μM/ml. Lysine Total[b]	L[c]	D[d]
Complete	21.2	10.5	10.3
Minus *P. vulgaris* cells	21.5	21.7	0.0
Minus L-lysine	0.0	0.0	0.0

[a] Ten ml. reaction mixture containing 35 mg. (dry weight) of washed cells and 220 μM of L-lysine in 0.1 M K_2HPO_4, adjusted to *p*H 8.4 with NH_4OH, was shaken with 0.2 ml. of toluene for 10 minutes and incubated at 28° for four hours. It was brought to *p*H 5.8 with H_3PO_4, held at 100° for 3 minutes, and centrifuged. [b] Quantitative paper chromatography in the following system: methyl ethyl ketone:acetic acid:water (90:25:30 by volume). [c] Manometric assay with L-lysine decarboxylase of *Bacterium cadaveris* NTCC 6578.[2] [d] Quantitative paper chromatography on residual lysine after complete decarboxylation in *c*.

The *p*H optimum of this reaction is 8.4. Similar results are obtainable by directly employing cells in their original culture broth. When a mixture of 10 g. of L-lysine hydrochloride and 100 ml. of fresh culture broth (containing approximately 4 g./l. dry weight of cells), adjusted to *p*H 8.4, was shaken with 2.5 ml. of toluene for 10 minutes and incubated at 28°, racemization was found to be complete in 16 hours. About 80% of the lysine was recovered from the mixture by absorption in IR-120 (NH_4^+ cycle), elution with 4% NH_4OH, and crystallization as the hydrochloride from aqueous ethanol. The product possessed negligible rotation, and was

(1) The medium contained corn steep liquor 20 g., $(NH_4)_2HPO_4$ 10 g., beet molasses 20 g., glycerol 10 g. and $MgSO_4 \cdot 7H_2O$ 1 g., adjusted to *p*H 7.5 with NH_4OH and made up to 1 l. with tap water. Two liters of this medium in a 4 liter fermenter was inoculated with *P. vulgaris* and incubated at 28° with stirring (1750 r.p.m.) and aeration (volume per volume per minute) for 16 hours.

(2) Purchased from Worthington Biochemical Corporation, Freehold, N. J. The assay was carried out as recommended in a one-side-arm flask, with the flask constant corrected for CO_2 retention.

shown by enzymatic and microbiological[3] assay to contain 50% L-lysine.

The conversion of D-lysine to L-lysine can be demonstrated manometrically by carrying out the racemization in the presence of L-lysine decarboxylase, as illustrated in Table II.

TABLE II

RACEMIZATION OF D-LYSINE BY LYOPHILIZED CELLS OF *Proteus vulgaris*

Reaction system	μM/flask CO_2 evolved	μM/flask Residual lysine[b]
Complete	16.5	2.7
Minus lyophilized cells	0.7[c]	20.1
Minus decarboxylase	0.0	21.0

[a] A Warburg flask containing 13 mg. of lyophilized cells and 20 μM of D-lysine in 2 ml. of 0.2 M potassium phosphate buffer (pH 5.8), and 5 mg. of L-lysine decarboxylase[2] in 0.5 ml. of buffer was incubated at 37° for 2.5 hours; the reaction was stopped by heating contents for 2 minutes at 100°. [b] Quantitative paper chromatography. [c] The sample of D-lysine contained (enzymatic assay) about 5% L-lysine.

The simplest explanation of these results is that *Proteus vulgaris* contains a lysine racemase.

(3) With *Leuconostoc mesenteroides* P-60.

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THE ADDITION OF HYDROGEN ATOMS TO SOLID OLEFINS AT −195°*

Sir:

The addition of H atoms to olefins in the gas phase at low temperatures is known to occur.[1] In an attempt to prepare free radicals in a condensed matrix, solid olefins were exposed at −195° to H atoms.[2] It was found that the H atom addition occurs and that the rate of hydrogen uptake depends strongly upon the olefin.

The experimental technique used in investigating this reaction consisted of exposing the olefin, uniformly deposited on the inner surface of a spherical, one-liter bulb immersed in liquid nitrogen, to the H atoms formed on an incandescent tungsten ribbon.[3] The olefin was introduced into an evacuated one-liter reaction vessel to a pressure of 2 mm. and condensed on the surface of a liquid nitrogen filled 2 cc. bulb, positioned near the center of the reaction vessel. The entire reaction vessel was immersed in liquid nitrogen and the refrigerant in the 2-cc. bulb evaporated. In this way the olefin was uniformly deposited on the reaction vessel walls. Pure hydrogen was introduced by diffusion through a heated palladium thimble. The tungsten ribbon centrally located in the vessel was heated to 1800° to produce H atoms. They reach the walls without recombining. The reaction with the olefin was followed by the pressure decrease.

* This research was performed under the National Bureau of Standards Free Radicals Research Program, supported by the Department of the Army.

(1) K. H. Geib and P. Harteck, *Ber.*, **66B**, 1815 (1933).

(2) F. O. Rice and M. Freamo, THIS JOURNAL, **75**, 548 (1953), have previously reported an attempt to hydrogenate a solid at liquid nitrogen temperature by irradiation with H atoms.

(3) I. Langmuir, *ibid.*, **34**, 1310 (1912); **36**, 417 (1915).

Rates of pressure decrease were observed for propylene, butene-1, isobutene, butadiene-1,3, pentene-1 and hexene-1. Thirty microns of hydrogen reacted completely with propylene in eight seconds. Butene-1 and isobutene reacted $^1/_3$ and $^1/_{20}$ as fast, respectively. Butadiene-1,3 and pentene-1 reacted very slowly and incompletely. Hexene-1 showed no measurable reaction. An analysis of the products of the hydrogen addition to butene-1 showed that *n*-butane, butene-2 and 3,4-dimethylhexane were formed. These results indicate that H atoms add to the terminal carbon of butene-1 to give secondary butyl radicals.[4] 3,4-Dimethylhexane results from dimerization while *n*-butane and butene-2 arise from a disproportionation reaction. H atom addition to butyl radicals to give *n*-butane cannot be excluded.

At least 80% of the propylene, butene-1 and isobutene could be hydrogenated at liquid nitrogen temperatures. It is apparent that considerable reaction has occurred throughout the bulk of the solid and that diffusion processes are operative. Either H atoms diffuse interstitially, or an H atom transfer from an alkyl radical to the olefin may effectively transport H atoms through the condensed phase.

This interpretation is valid provided the olefin does not reach the hot tungsten ribbon. A control experiment was performed using butene-1 and helium instead of hydrogen. The initially deposited butene-1 was the only hydrocarbon found after warm-up. It can be concluded that heat transfer from the ribbon to the surface was insufficient to evaporate the butene-1.

There is no doubt that H atom addition to some solid olefins can occur at −195°. The analytical results indicate clearly that alkyl radicals were formed. It cannot yet be stated whether these radicals are stabilized in an olefin matrix and undergo reaction on warm-up, or that they exist only in small stationary state concentrations.

(4) W. J. Moore and L. A. Wall (*J. Chem. Phys.*, **17**, 1335 (1949)) obtained similar results from a mercury sensitized hydrogenation of butene in the gas phase.

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RECEIVED DECEMBER 17, 1957

THE STRUCTURE OF CITROSTADIENOL, A NATURAL 4α-METHYLSTEROL

Sir:

The isolation of citrostadienol [$C_{30}H_{50}O$ ± CH_2; m.p. 162–164°, $[\alpha]_D$ +24° (all rotations in chloroform)] from Israeli grapefruit and orange peel-oil was reported recently.[1] The substance (a companion of β-sitosterol) appeared to be a doubly unsaturated 3β-hydroxy-steroid, except that the optical rotation data resembled those of the tetracyclic triterpenes rather than the steroids. We have now shown citrostadienol to be 4α-methyl-$\Delta^{7,24(28)}$-stigmastadien-3β-ol (4α-methyl-24-ethylidene-Δ^7-cholesten-3β-ol) (I).

(1) A. Weizmann and Y. Mazur, *J. Org. Chem.*, in press.