lent weights found, 228.7 and 229.9; average, 229.3; calculated on the basis of $C_{29}H_{50}O_4$, 231.2.

Summary

1. A series of derivatives of spinastanol have been prepared, the physical constants of which warrant the conclusion that this saturated sterol is identical with fucostanol and stigmastanol.

2. The identity of spinastanol, fucostanol and stigmastanol points out the isomerity of the naturally occurring sterols from which these saturated derivatives are obtained.

3. Spinastanol appears to differ from ostreastanol and sitostanol, probably in the arrangement of the side chain rather than in the carbon skeleton of the nucleus.

4. Hydrolysis of the acetyl and benzoyl esters of spinastanol, and titration of the equivalent weight of spinastanedicarboxylic acid, establish for the spinach sterols a skeleton of twenty-nine carbon atoms, in common with other well investigated phytosterols.

ROCHESTER, N. Y. RECEIVED JULY 25, 1938

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, NO. 174]

Studies in Organic Peroxides. V. *t*-Butyl Hydroperoxide[1]

BY NICHOLAS A. MILAS AND S. ARTHUR HARRIS[2]

The extraordinary stability of *t*-butyl hydrogen peroxide solution[3] and its therapeutic efficacy in the treatment of various fungus diseases[4] have stimulated our interest in the investigation of its chemical nature. In view of the lability of the hydroxyl group in tertiary alcohols, it was early suspected that pure hydrogen peroxide in non-aqueous solution would react with *t*-butyl alcohol reversibly to form *t*-butyl hydroperoxide. Although such a reaction between alcohols and hydrogen peroxide is unknown, an analogous reaction between organic acids and hydrogen peroxide in non-aqueous solvents has been known for some time.[5]

$$(CH_3)_3COH + HOOH \rightleftarrows (CH_3)_3COOH + H_2O$$

All of the alkyl hydroperoxides known, methyl,[6] ethyl,[6] isopropyl,[7] are much less stable than *t*-butyl hydroperoxide, which, besides being stable at room temperature, decomposes very slowly in alkalies or in the presence of pure liver catalase or horse-radish peroxidase. A distinct advantage, however, lies in the simplicity of its preparation. While the other hydroperoxides have been made by the alkylation of hydrogen peroxide with the corresponding dialkyl sulfates, *t*-butyl hydroperoxide can be prepared very easily from the anhydrous *t*-butyl alcoholic solution of hydrogen peroxide[3] by subjecting the latter to fractionation under reduced pressure in the presence of dehydrating agents such as anhydrous magnesium sulfate or preferably glacial metaphosphoric acid.

Experimental Part

Preparation of *t*-Butyl Hydroperoxide.—To 600 cc. of 30% aqueous hydrogen peroxide "Albone C" was added with frequent shaking 2340 cc. of *t*-butyl alcohol (b. p. 81.7–81.8°) and the solution allowed to stand for fifteen minutes, when 225 g. of anhydrous sodium sulfate was added slowly with vigorous shaking. The mixture separated into two layers and the non-aqueous layer containing most of the peroxide was removed and shaken first with 225 g. more of anhydrous sodium sulfate, then with two 225-g. portions of anhydrous magnesium sulfate. The final mixture which was essentially free from water was filtered and the filtrate allowed to stand several days over 400 g. of glacial metaphosphoric acid. This treatment produced a peroxide solution having an active oxygen content equivalent to about 17% *t*-butyl hydroperoxide.

To obtain the pure hydroperoxide, samples of the above were fractionated several times under diminished pressure over glacial metaphosphoric acid or anhydrous magnesium sulfate using an all-glass apparatus and the fraction boiling at 38–38.5° (18 mm.) collected and analyzed. Both the metaphosphoric acid and the magnesium sulfate, when used as dehydrating agents, brought about the production of the same peroxide. That this peroxide is not a constant boiling mixture between hydrogen peroxide and *t*-butyl alcohol, is shown by its physical and chemical properties.

Anal. Calcd. for $C_4H_{10}O_2$: C, 53.33; H, 11.11; active (O), 17.78. Found: C, 53.68, 53.63; H, 11.10, 11.17; active (O), 17.84, 18.10, 18.21.

(1) For other papers in this series see THIS JOURNAL, **55**, 349, 352 (1933); **56**, 1219, 1221 (1934).

(2) Fellow of the Massachusetts Pharmaceutical Corporation.

(3) Milas and Sussman, THIS JOURNAL, **58**, 1302 (1936).

(4) Combes, *N. Y. State J. Med.*, **37**, No. 22 (1937).

(5) D'Ans and Frey, *Ber.*, **45**, 1845 (1912); Hatcher and Sturrock, *Can. J. Research*, **4**, 35 (1931).

(6) Baeyer and Villiger, *Ber.*, **33**, 3387 (1900); **34**, 738 (1901); Rieche and Hitz, *ibid.*, **61**, 951 (1928); **62**, 218, 2458 (1929); Rieche, "Alkylperoxide und Ozonide," Theodor Steinkopff, Dresden, 1931, and "Die Bedeutung der organische Peroxyde für die chemische Wissenschaft und Technik," Ahrens Sammlung, **34**, 1 (1936), Ferdinand Enke, Stuttgart.

(7) Medwedew and Alexejewa, *Ber.*, **65**, 133 (1932).

The peroxide is a colorless ethereal liquid with a sharp irritating odor having a density at 25° of 0.91063 and a freezing point of −13.5°. It is soluble in most of the ordinary organic solvents and unlike hydrogen peroxide is completely soluble in saturated hydrocarbons such as petroleum ether; in water it goes into solution slowly. It explodes violently when heated in the open flame although it has been refluxed for several hours in *t*-butyl alcohol without any appreciable decomposition. It liberates iodine slowly from an acidified solution of potassium iodide; it decomposes rapidly when heated with dilute alkalies, but retains its peroxide strength for several months when mixed at room temperature with a 10% solution of sodium hydroxide.

t-Butyl hydroperoxide is not easily converted into *t*-butyl chloride unless a reducing agent is used together with concentrated hydrochloric acid. Thus, when an ice-cold solution of concentrated hydrochloric acid containing 20 g. of ferrous chloride was mixed with about 4 g. of pure *t*-butyl hydroperoxide, an immediate reaction occurred with the separation of *t*-butyl chloride, which was separated, shaken with sodium bicarbonate solution, dried over calcium chloride and distilled; a yield of 2.5 g. of *t*-butyl chloride, b. p. 51°, was obtained.

The Effect of Catalase on the Decomposition of *t*-Butyl Hydroperoxide.—It has been known for some time that catalase has a specific action on the decomposition of hydrogen peroxide but has no effect on alkyl hydroperoxides.[8] Contrary to these early observations Stern[9] recently has reported that ethyl hydroperoxide is slowly attacked by catalase although Keilin and Hartree[10] are not in agreement with this observation and attributed the decomposition of this peroxide to a secondary reaction.

Catalase has very little or no effect on the decomposition of *t*-butyl hydroperoxide as can be seen readily by the results given in Table I. The catalase used in these experiments was a crystalline preparation[11] suspended in ammonium sulfate solution and kindly supplied to us by Professor Sumner of Cornell University. The *p*H of the catalase was adjusted just before use with disodium phosphate to 8.2[12] and 1 cc. of this solution was added to each of several glass-stoppered tubes. By means of a calibrated 0.1-cc. special pipet, 0.1 cc. of *t*-butyl hydroperoxide was added to each tube except one which was reserved for a blank with 0.1 cc. of 30% aqueous hydrogen peroxide solution. All tubes were shaken during the experiments at about 26.5°. At the end of a definite time the entire contents of each tube was treated with an acidified solution of sodium iodide and the iodine liberated titrated against standard thiosulfate solution. The hydrogen peroxide in the blank was destroyed completely by the catalase preparation in less than two minutes. With *t*-butyl hydroperoxide, on the other hand, the catalase turned red from a straw color and back again to its original color after fifteen hours of shaking with no evolution of gas. This color change may indicate the formation of an intermediate complex between the peroxide and catalase. An analogous observation was made by Stern[9b] with ethyl hydroperoxide. The results of seven experiments are shown in Table I.

TABLE I

EFFECT OF CATALASE ON THE DECOMPOSITION OF *t*-BUTYL HYDROPEROXIDE

Time, min.	0	3	9	12	30	60	930
0.01982 *N* thio, cc.	71.0	68.6	66.0	67.5	70.0	68.2	68.0

Effect of Peroxidase on the Decomposition of *t*-Butyl Hydroperoxide.—Peroxidase, unlike catalase, has been found to activate the decomposition of alkyl hydroperoxides in the presence of acceptors such as pyrogallol which is simultaneously oxidized to purpurogallin.[13] Although the decomposition of *t*-butyl hydroperoxide is activated slowly by peroxidase, we failed to observe any precipitate of purpurogallin. The peroxidase preparation used in our experiments was an extract of horse-radish kindly supplied to us by Professor Gould of our Biology Department. The *p*H of the preparation was adjusted to 6.21 and all of the experiments were carried out at 28°.

The experimental procedure was as follows: 2 g. of pure pyrogallol was dissolved in 800 cc. of distilled water and to each of several 250-cc. glass-stoppered Erlenmeyer flasks was added 80 cc. of this solution, 5 cc. of the peroxidase preparation and 0.1 cc. of *t*-butyl hydroperoxide; one sample was retained as a blank to which was added 0.1 cc. of 30% hydrogen peroxide instead of *t*-butyl hydroperoxide. At the end of a definite time each sample was titrated as before for the presence of peroxide. Hydrogen peroxide was destroyed completely in about four minutes with considerable evolution of heat and abundant precipitation of purpurogallin. In the experiments with *t*-butyl hydroperoxide, the solution turned orange in color but no precipitate appeared even after one hour of standing. The peroxide was used up very slowly, as the results indicate in Table II.

TABLE II

EFFECT OF PEROXIDASE ON THE DECOMPOSITION OF *t*-BUTYL HYDROPEROXIDE

Time, min.	0	5	10	60
0.01982 *N* thio, cc.	71.5	61.2	63.0	60.6

Effect of Palladium Black on the Decomposition of *t*-Butyl Hydroperoxide.—The palladium black used in these experiments was prepared in accordance with the method of Wieland.[14] Ten mg. of this catalyst was mixed in each of several experiments with 1 cc. of disodium phosphate solution (*p*H 8.2) containing 0.1 cc. of *t*-butyl hydroperoxide and the mixture shaken at room temperature in glass-stoppered tubes similar to those used with the catalase experiments. The results of four experiments are shown in Table III. Hydrogen peroxide under the same conditions was destroyed completely by our palladium catalyst in less than one minute.

(8) Bach and Chodat, *Ber.*, **36**, 1756 (1903).

(9) Stern, (a) *J. Biol. Chem.*, **114**, 473 (1936); (b) *Enzymologia*, **4**, 145 (1937).

(10) Keilin and Hartree, *Proc. Roy. Soc.* (London), **B121**, 172 (1937).

(11) Sumner and Dounce, *Science*, **85**, 366 (1937); *J. Biol. Chem.*, **121**, 417 (1937).

(12) Michaelis [*Biochem. Z.*, **53**, 320 (1912)] found that the catalytic activity of catalase was best at *p*H range 7–9.

(13) Bach and Chodat, *Ber.*, **36**, 604 (1903); Wieland and Sutter, *ibid.*, **63**, 73 (1930).

(14) Wieland, *ibid.*, **45**, 489 (1912).

TABLE III

EFFECT OF PALLADIUM ON THE DECOMPOSITION OF *t*-BUTYL HYDROPEROXIDE

Time, min.	0	10	23	52
0.01982 *N* thio, cc.	66.0	38.0	22.0	21.7

The results in Table III seem to indicate that palladium black slowly accelerates the decomposition of *t*-butyl hydroperoxide in a dilute solution of disodium phosphate. However, pure *t*-butyl hydroperoxide is only slowly attacked by palladium black and a pure sample of it stirred over this catalyst for several months was still strongly peroxidic.

Summary

1. *t*-Butyl hydroperoxide has been prepared by the fractionation of an anhydrous solution of hydrogen peroxide in *t*-butyl alcohol in the presence of dehydrating agents.

2. This peroxide has been found to be very stable under ordinary conditions.

3. Pure liver catalase and horse-radish peroxidase have very little or no effect on the decomposition of *t*-butyl hydroperoxide.

4. Palladium black catalyzes slowly the decomposition of *t*-butyl hydroperoxide in dilute solution of disodium phosphate, although the decomposition of the peroxide is only slightly affected by this catalyst.

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[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF JOHNS HOPKINS UNIVERSITY]

Lengthening Carbon Chains by Three Units: Assay of Primary Bromides from the Addition of Hydrogen Bromide

BY A. P. KOZACIK AND E. EMMET REID

It is well known that an alkyl bromide may be converted into a primary alcohol with either one or two additional carbon atoms in the chain. The object of this investigation was to provide a method of lengthening a carbon chain by three $-CH_2-$ units: $RBr \longrightarrow RMgBr \longrightarrow RCH_2CH{=}CH_2 \longrightarrow RCH_2CH_2CH_2Br$. This combines an old reaction with one[1] that has been studied only recently. In addition to lengthening the chain this gives odd-numbered bromides from the readily obtainable even. It was particularly interesting to extend these reactions to the higher alkyls and to aromatics. Another object was to identify the primary bromides by crystalline derivatives and to get a rough estimate of their purity. The abnormal addition of hydrogen bromide is of great theoretical interest; the availability of the primary bromides so produced for syntheses is a practical question of importance. The alkenes are given in Table I.

The addition of the hydrogen bromide was carried out by Professor Kharasch.[2] Our results show conclusively that the primary bromides are obtained. The melting points of these are in Table II. It was too much to expect that the technique which he has developed for the lower alkenes would apply to the higher without some modification. Unfortunately the whole amounts of the alkenes that were available were run through with the standard technique before any of the products were tested. The results were good with nonene-1 but very poor for heptadecene-1 and nonadecene-1. The identity of the synthetic bromides was shown and their approximate purity estimated by their reaction with para substituted phenols. For compari-

TABLE I

PROPERTIES OF THE ALKENES

Alkenes	B. p., °C.	Press., mm.	M. p., °C.	d^0_4	d^{25}_4	$n^{25}D$	Bromine no. Calcd.	Found		Yield, %
n-$C_{11}H_{23}CH{=}CH_2$	102–103	10	−13	0.7856	0.7670	1.4328	62.8	63.6	63.7	77
n-$C_{13}H_{27}CH{=}CH_2$	127.5–128.5	10	− 2.8	.7921	.7751	1.4353	55.7	54.5	54.6	67
n-$C_{15}H_{31}CH{=}CH_2$	155.4–156.4	10	11.2	$.7892^{20}_4$	.7859	1.4417	49.7	47.7	48.5	43
n-$C_{17}H_{35}CH{=}CH_2$	177	10	21.7	$.7889^{20}_4$	$.7858^{25}_4$		46.9	45.7		56
$C_6H_5(CH_2)_2CH{=}CH_2$	181–182	757								77
$C_6H_5(CH_2)_4CH{=}CH_2$	94.5–95	10								87

(1) Kharasch and co-workers, THIS JOURNAL, **55**, 2469, 2521 and 2531 (1933).

(2) M. S. Kharasch and Wm. M. Potts, *J. Org. Chem.*, **2**, 195 (1937).