by Wieland and co-workers[2] in the determination of very small quantities (concentrations) of hydrogen peroxide formed during the oxidation of various organic substances by oxygen in aqueous solution. It seemed probable that cerous hydroxide might react likewise with peroxides present in many ethers.

Recently Moffett and Aspergen[3] reported an unexpected fire which resulted from addition of lithium aluminum hydride to tetrahydrofuran which was presumed to be free from peroxides but shown later to contain them. For removing peroxides from ethers they stress the need and value of having a solid substance which is insoluble and which can therefore be separated from the ether by filtration or decantation. Our results show that cerous hydroxide, $Ce(OH)_3$, fulfills these requirements.

Experimental Procedure and Results

Cerous hydroxide is prepared readily by precipitation from a cerous salt solution (cerous chloride used) by addition of aqueous sodium hydroxide until the supernatant solution is slightly alkaline. The cerous hydroxide (white) is easily removed and washed by centrifugation. The undried cerous hydroxide is used since it is doubtful whether the thoroughly dried hydroxide would react as rapidly with peroxides.

If peroxides are present the cerous hydroxide changes color (from white to reddish-brown) within a minute or two after addition to the ether (30 ml. used). With those ethers which are miscible with water to an appreciable extent the time required to remove the peroxides present (as shown by the iodide test) is in no case greater than 15 minutes. For ethers which are only slightly miscible with water it is necessary to add one or two milliliters of water along with the cerous hydroxide in order to cause complete removal of peroxides in 15 minutes. Otherwise, after partial reaction with peroxides has occurred, the colored precipitate becomes agglomerated in such a way as to prevent rapid reaction between the cerous hydroxide and the peroxides remaining. When water is not added in these cases from three to four hours are required for complete peroxide removal. Incidentally, the use of starch–iodide paper in testing for the presence of peroxides in ethers is not as reliable as a slightly acidified solution of potassium iodide containing starch.

The peroxyceric compound and unchanged cerous hydroxide is removed from the ether by centrifugation and decantation. The cerium present in this mixture may be recovered as cerous cerium, Ce^{III}, by simply dissolving in hydrochloric acid and reducing the Ce^{IV} present to Ce^{III} by addition of the required amount of "Superoxol" (30% hydrogen peroxide), which is shown by the disappearance of the yellow color of Ce^{IV}. Heating gently for a short time assures complete removal of excess hydrogen peroxide. Cerous hydroxide is slowly oxidized by oxygen of the air and if it is to be kept for considerable time it should be stored in an air-tight container. An evacuated desiccator, containing no desiccant, has been found quite satisfactory. In general there should be no need for storage since it is so easily prepared when needed.

After the removal of the peroxides the ethers are tested for the presence of cerium by the benzidine test.[4] The limiting quantity of cerium (as Ce^{III} or Ce^{IV}) identifiable by this test is reported to be 0.18 γ and the limiting concentration, 1 part in 275,000. Of the nineteen ethers used only allyl ethyl ether and benzyl *n*-butyl ether gave a positive test for cerium; the latter very slightly positive and the former, strongly positive. The allyl ethyl ether was distinctly yellow, thus indicating the presence of considerable cerium. This may be attributable to the formation of a complex between ceric cerium and this ether (due to the presence of an active double bond), thereby forming a cerium compound which is appreciably soluble in the ether.

The ethers tested (listed below) were of the highest grade commercially available. The quantities of peroxide found present, as indicated by the iodine liberated in the iodide test (2 ml. of the ether +5 ml. of acidified potassium iodide solution +1 ml. of starch solution), are designated by 0 for none, + for very small, ++ for moderate and +++ for considerable or large.

Allyl ethyl ether, ++	*p*-Dioxane, ++
Allyl phenyl ether, ++	Ethyl ether,[a] 0
Benzyl ether, ++	Ethyl ether,[b] +++
Benzyl *n*-butyl ether, ++	Ethyl ether,[c] ++
o-Bromophenetole, +	Isopropyl ether, +++
p-Bromophenetole, 0	*o*-Methylanisole, 0
n-Butyl ether, ++	*m*-Methylphenetole, 0
t-Butyl ether, ++	Phenetole, 0
p-Chloroanisole, 0	Tetrahydrofuran, ++
o-Chlorophenetole, 0	

Diethylene glycol diethyl ether, +++
Diethylene glycol mono-*n*-butyl ether, ++

[a] Obtained from sealed tin can of anhydrous ether, analytical reagent, immediately after opening. [b] Obtained from a partially filled tin can (well-stoppered) containing the same grade of anhydrous ether, originally, as that described in note *a*, but of long standing. [c] From a galvanized iron container used for dispensing ether from stockroom for research purposes.

After treatment with cerous hydroxide each of the peroxide-containing ethers, listed above, gives a negative test for peroxide. Also the benzidine test for cerium in the resulting peroxide-free ether was negative in every case with the exception of the two discussed above.

It may be of interest to note that di-*t*-butyl peroxide does not liberate iodine from acidified potassium iodide solution nor does it react with cerous hydroxide. The peroxides found present in *t*-butyl ether are, therefore, other more reactive peroxides.

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(2) H. Wieland and B. Rosenfeld, *Ann.*, **477**, 32 (1929).

(3) R. B. Moffett and B. D. Aspergen, *Chem. Eng. News*, **32**, 4328 (1954).

(4) F. Feigl, "Qualitative Analysis by Spot Tests," 3rd Ed., translated by R. E. Oesper, Elsevier Publ. Co., New York, N. Y., 1946, p. 161.

Phenylglyoxylates of Steroid Alcohols[1]

BY HANS REICH[2]

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Steroid ketones can be converted readily to 2,4-dinitrophenylhydrazones and estimated spectrophotometrically. If a similar method could be found for steroid alcohols, it would facilitate their estimation in biological material and incubation mixtures. It would require first the formation of a keto acid ester, for instance a *d*-camphor 10-sulfonate. Such an ester was prepared from cholesterol, but it was found that it gave only a poor yield of a 2,4-dinitrophenylhydrazone due to the fact that the keto group in camphor is rather unreactive. Better results were obtained with the phenylglyoxylic acid esters of cholestane-3β-ol[3,4] and cholesterol which can be prepared readily in good yield. They reacted quantitatively with dinitrophenylhydrazine in a mixture of ethanol and chloroform containing a small amount of hydrochloric acid. Under the same conditions dehydroepiandrosteryl phen-

(1) This work was supported in part by research grants from the National Cancer Institute of the National Institutes of Health, Public Health Service; from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council; and from Armour and Company, Chicago, Ill.

(2) Biochemistry Department, College of Agriculture, University of Wisconsin, Madison, Wis.

(3) W. G. Dauben, D. F. Dickel, O. Jeger and V. Prelog, *Helv. Chim. Acta*, **36**, 325 (1953).

(4) This material was kindly supplied by Dr. V. Prelog, Zurich, Switzerland.

ylglyoxylate consumed two moles of dinitrophenylhydrazine. The dinitrophenylhydrazones thus obtained were quite pure and not contaminated with ethyl phenylglyoxylate which could have been formed by transesterification. Hydrolysis was accomplished by refluxing with potassium bicarbonate in aqueous methanol. The dinitrophenylhydrazone of phenylglyoxylic acid and the free steroid alcohols which resulted from this fission were obtained in quantitative yields. The phenylglyoxylate dinitrophenylhydrazones of cholesterol, cholestane-3β-ol and (−)-neomenthol[4] showed a maximum at 387–388 mμ, that of dehydroepiandrosterone at 372 mμ.

Experimental[5]

Ethyl *d*-Camphor 10-Sulfonate Dinitrophenylhydrazone.—The crude product was chromatographed, eluted with mixtures of benzene and chloroform, and crystallized from chloroform–ethanol; m.p. 156–158°, λ Chf 362 mμ.

Anal. Calcd. for $C_{18}H_{24}O_7N_4S$: N, 12.72. Found: N, 12.82.

Cholesteryl *d*-Camphor 10-Sulfonate.—To a cooled solution of 64 mg. of cholesterol in 0.5 cc. of pyridine 71.4 mg. of *d*-camphor 10-sulfonyl chloride was added. The mixture was kept in the cold room overnight. After addition of ice and standing at room temperature for 1 hr., the crystals were filtered, washed with water, dried and recrystallized twice from ether–methanol. The ester formed white leaflets, m.p. 162–164.5°.

Anal. Calcd. for $C_{37}H_{60}O_4S$: S, 5.34. Found: S, 5.30.

Cholesteryl *d*-Camphor 10-Sulfonate Dinitrophenylhydrazone.—A solution of 45 mg. of cholesteryl *d*-camphor 10-sulfonate in 1 cc. of absolute ethanol and 0.5 cc. of chloroform was mixed with a solution of 15 mg. of dinitrophenylhydrazine in 1.8 cc. of absolute ethanol and 4 drops of concentrated hydrochloric acid. After standing at room temperature for 37 hours, the excess reagent was converted to pyruvic acid dinitrophenylhydrazone[6] which was removed by extraction with sodium carbonate. The neutral dinitrophenylhydrazone, crystallized from chloroform–ethanol, melted at 97–102°.

Anal. Calcd. for $C_{43}H_{64}O_7N_4S$: N, 7.18. Found: N, 6.77.

Cholestane-3β-yl Phenylglyoxylate Dinitrophenylhydrazone.—A solution of 27.4 mg. of dinitrophenylhydrazine in 3 cc. of absolute ethanol and 6 drops of concentrated hydrochloric acid was mixed with a solution of 29.6 mg. of cholestane-3β-yl phenylglyoxylate in 3 cc. of chloroform. The reagent utilized after 1 hr. amounted to 0.95 mole. The neutral dinitrophenylhydrazone was chromatographed and eluted with hexane–benzene (2:3 to 1:4). It crystallized from chloroform–ethanol in yellow granules, m.p. 244–246.5°, λ^{Chf} 387 mμ.

Anal. Calcd. for $C_{41}H_{56}O_6N_4$: N, 8.00. Found: N, 8.14.

The same derivative was obtained, when the reaction was carried out in chloroform–acetic acid.[6]

Hydrolysis was accomplished by refluxing 27.9 mg. of dinitrophenylhydrazone and 140 mg. of potassium bicarbonate in a mixture of 6 cc. of methanol and 3.2 cc. of water for 23 hr. The acidic material was separated and the hydrolysis repeated. The phenylglyoxylic acid dinitrophenylhydrazone isolated amounted to 13.0 mg. (calculated 13.15 mg.). After recrystallization from aqueous acetic acid, it melted at 202–203.5° (lit.[7] m.p. 196–197°). The neutral material weighed 15.2 mg. (calculated 15.5 mg.). After chromatography and recrystallization from 95% ethanol, cholestane-3β-ol, m.p. 143–144°, was obtained.

(5) Microanalyses by Huffman Microanalytical Laboratories, Wheatridge, Colo. All melting points were observed on a Kofler hot-stage. Acid-washed Alcoa aluminum oxide was used for chromatography. The ultraviolet spectra were taken in chloroform.

(6) H. Reich, K. F. Crane and S. J. Sanfilippo, *J. Org. Chem.*, **18**, 822 (1953).

(7) B. B. Corson, N. E. Sanborn and P. R. Van Ess, THIS JOURNAL, **52**, 1623 (1930).

Cholesteryl Phenylglyoxylate.—A mixture of 0.2 cc. of phenylglyoxylic acid chloride and 1 cc. of absolute benzene was added to a solution of 200 mg. of cholesterol in 1 cc. of absolute pyridine and 1.5 cc. of absolute benzene. After standing overnight, ice was added, followed by extraction with hexane. The solutions were washed to neutrality, dried and chromatographed. The fractions eluted with hexane and hexane–benzene (to 7:3) were crystallized from ether–methanol and gave white leaflets, m.p. 120–122°.

Anal. Calcd. for $C_{35}H_{50}O_3$: C, 81.03; H, 9.72. Found: C, 81.07; H, 9.81.

Cholesteryl Phenylglyoxylate Dinitrophenylhydrazone.—This compound was prepared in the same manner as the corresponding cholestanyl derivative. The dinitrophenylhydrazine utilized amounted to 0.94 mole. The dinitrophenylhydrazone, after chromatography (eluted with hexane–benzene 1:1 to 1:4) and crystallization from chloroform–ethanol, melted at 233–235°, λ^{Chf} 387 mμ.

Anal. Calcd. for $C_{41}H_{54}O_6N_4$: N, 8.02. Found: N, 8.20.

When the reaction was carried out in chloroform–acetic acid, more than the calculated amount of dinitrophenylhydrazine was utilized. The reaction product was contaminated with a red dinitrophenylhydrazone and difficult to purify.

Dehydroepiandrosteryl Phenylglyoxylate.—This ester was prepared from 150 mg. of dehydroepiandrosterone and 0.4 cc. of phenylglyoxylic acid chloride as described above. After chromatography (eluted with mixtures of hexane and benzene) and crystallization from chloroform–methanol, it melted at 186–187°.

Anal. Calcd. for $C_{27}H_{32}O_4$: C, 77.11; H, 7.67. Found: C, 76.63; H, 8.04.

Dehydroepiandrosteryl Phenylglyoxylate Bisdinitrophenylhydrazone.—A solution of 16.4 mg. of the phenylglyoxylate in 4 cc. of chloroform was added to a solution of 31.0 mg. of dinitrophenylhydrazine in 4 cc. of absolute ethanol and 8 drops of concentrated hydrochloric acid. The mixture was worked up as described above. The reagent utilized amounted to 1.88 moles. The dinitrophenylhydrazone was chromatographed, eluted with mixtures of benzene and chloroform, and crystallized from chloroform–ethanol. It melted at 264–266° dec., λ^{Chf} 372 mμ.

Anal. Calcd. for $C_{39}H_{40}O_{10}N_8$: N, 14.35. Found: N, 14.04.

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Furo-chromones and -Coumarins (X): On the Constitution of Prangenin

BY ALEXANDER SCHÖNBERG AND GAMIL AZIZ

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Pigulevskii and Kuznetsova[1] have isolated from *Prangos pabularia* a compound which they believe to be a substance of the constitution (Ia)[2] which they named prangenin.

Formula Ia shows a butyl ether of xanthotoxol and the aim of the investigation is to establish which of the four possible isomers, normal, iso, *sec-* or *t*-butyl ether is identical with prangenin, m.p. 97°.

Schönberg and Sina[3] have described the *n*-butyl ether, m.p. 83°; this therefore is not identical with prangenin.

The authors allowed xanthotoxol (Ib) to react in acetone with *sec*-butyl iodide and with isobutyl io-

(1) G. V. Pigulevskiĭ and G. A. Kuznetsova, *Zhur. Obshchei Khim.*, **23**, (7), 1237 (1953).

(2) Unfortunately the abstract of the Russian paper (*C. A.*, **47**, 12341f (1953)) is not free from errors: in structure (I) "Bu" should be replaced by "C_4H_9" and "IV" next to the last line should read (II).

(3) A. Schönberg and A. Sina, THIS JOURNAL, **72**, 4826 (1950).