TABLE I

INHIBITORY ACTIVITY OF PURE COMPOUNDS ON GROWTH OF TOMATO SEEDLINGS

Substance	Concn. needed for 50% inhibition in height growth (mg./l.)	No. of plants dead after 1 week 500 mg./l.	250 mg./l.
Benzene	>1000	0	0
Anisole	>1000	0	0
Benzaldehyde	165	7	3
Acetophenone	365	1	0
p-Methoxyacetophenone	145	10	0
o-Methoxybenzaldehyde	170	6	4
m-Acetylbenzaldehyde	140	7	4
3-Acetyl-6-methoxybenzaldehyde	127	10	8
3-Acetyl-6-methoxybenzonitrile	90	10	10
3-Amino-4-methoxyacetophenone	40	10	10
4-Methoxy-3-nitroacetophenone	45	10	10
3-Acetyl-6-methoxybenzoic acid	237	7	2
4-Methoxyisophthalic acid	270	1	0
2-Methoxy-5-methylbenzaldehyde	125	10	5
Phenol	225	2	0
Salicylaldehyde	125	9	0
p-Hydroxyacetophenone	130	10	3
o-Hydroxyacetophenone	325	0	0
o-Methoxyacetophenone	175	8	0
3-Acetyl-4-hydroxyacetophenone	60	10	0
Aniline	155	0	0
Nitrobenzene	100	0	0
Benzoic acid	150	10	3

(3-acetyl-6-methoxybenzaldehyde) which contains all three groups is more toxic than combinations of any two of the other groups. The substitution of a cyano, nitro, or amino group for the aldehyde group causes increased inhibition, the amino group having the most toxic effect. The last two columns in the table show that most of these compounds do not cause death of the plants even though they may cause more inhibition in height growth than the naturally occurring inhibitor. Only the nitro, cyano, and amino substituted analogs brought about as high a mortality as the natural substance itself.

Acknowledgment.—The authors wish to express their appreciation to Dr. E. R. Buchman and to Dr. David R. Howton, Gates and Crellin Laboratories of Chemistry, California Institute of Technology, for their advice, assistance and encouragement in the prosecution of this work.

Summary

1. The structure determination and synthesis of a new compound, 3-acetyl-6-methoxybenzaldehyde, which was isolated from the leaves of *Encelia farinosa* is given.

2. The inhibitory activity of the toxic compound and other related compounds on the growth of tomato seedlings in solution culture is demonstrated.

PASADENA, CALIF. RECEIVED NOVEMBER 15, 1947

[CONTRIBUTION FROM THE UPJOHN RESEARCH LABORATORIES]

Crystalline Vitamin A Methyl Ether[1a]

BY A. R. HANZE, T. W. CONGER, E. C. WISE* AND D. I. WEISBLAT

The increased interest in vitamin A syntheses has placed vitamin A methyl ether in a position of considerable prominence, since its synthesis should be less difficult than that of the alcohol or its esters. The synthesis of vitamin A ethers recently has been the subject of numerous papers and patents.

Kipping and Wild[1] were the first to outline a possible synthesis of vitamin A methyl ether but no further work has appeared giving the results of this proposed synthesis. Oroshnik[2] reported a synthesis of vitamin A methyl ether which gave two fractions whose absorption maxima differed from that of vitamin A by 10 and 13 mμ. No methoxyl analysis or biological assay of these fractions has been published. Milas[3] has published the synthesis of a product having a reproducible activity of 1.5 to 3.0% that of vitamin A. Cornwell[4] reported a synthesis of an ether of vitamin A, identifying the product by spectrophotometric data only. Isler, *et al.*,[5] reported the synthesis of a product having a potency greater than that of β-carotene.

In spite of the large amount of work done on vitamin A methyl ether, no one had reported the preparation of a product of sufficient purity for the property determination of its properties. For this reason we undertook the synthesis of pure vitamin A methyl ether and in a preliminary report have given[6] its physical properties and biological potency. The complete experimental details as to its preparation as well as the behavior of vitamin a methyl ether under various experimental conditions are given here.

* Deceased December 7, 1947.

(1) Kipping and Wild, *Chem. and Ind.*, **58**, 802 (1939).

(1a) Presented before the Division of Organic Chemistry, 112th A. C. S. Meeting, New York, N. Y., September 17, 1947.

(2) Oroshnik, THIS JOURNAL, **67**, 1627 (1945).

(3) Milas, *Science*, **103**, 581 (1946); this article contains references to the author's numerous patents in this field.

(4) Bishop C. Cornwell, U. S. Patent 2,414,722 (January 21, 1947).

(5) Isler, *et al.*, *Experientia*, **2**, 31 (1946); Jubilee Vol. Emil Barell, 31–44 (1946).

(6) Hanze, Conger, Wise and Weisblat, THIS JOURNAL, **68**, 1389 (1946).

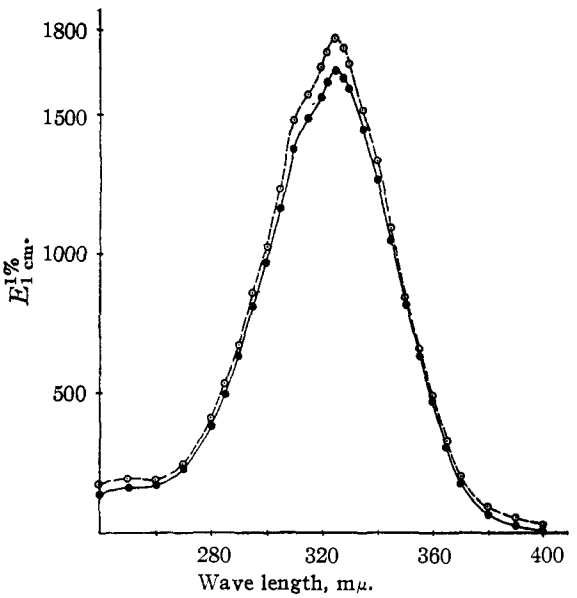


Fig. 1.—Spectrophotometric curves of vitamin A methyl ether, ●, and of vitamin A alcohol, ⊙, in isopropanol.

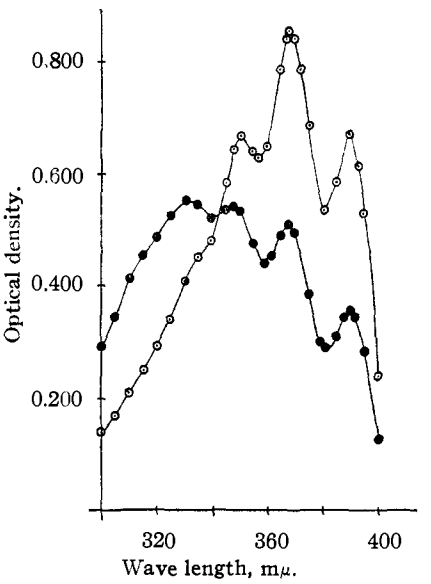


Fig. 2.—Spectrophotometric curves in isopropanol of vitamin A methyl ether, ●, and of vitamin A alcohol, ⊙, after treatment with 0.033 *N* hydrogen chloride in ethanol.

Subsequent to our initial publication[6] Cawley[7] reported the preparation of a vitamin A methyl ether for which he records a melting point of 33–33.5°, an extinction coefficient $E^{1\%}_{1\text{ cm.}}$ at 328 mμ of 1800 and a biological potency of 4,800,000 units per gram.

Vitamin A methyl ether was prepared by the action of dimethyl sulfate on the lithium derivative of the vitamin. The lithium derivative was prepared by the reaction of *n*-butyl lithium[8] with crystalline vitamin A alcohol.[9] Attempts were made to form a metallic vitaminate using triphenylmethyl sodium, a Grignard reagent or potassium metal. The former possesses the disadvantage of giving triphenylmethane which is extremely difficult to separate from the desired product, while the latter two destroy the vitamin A molecule, the Grignard reagent with the formation of a product with absorption maxima at 333, 348 and 366 mμ and the metallic potassium generating hydrogen which probably reduces the molecule. Milas[10] has reported the preparation of metallic salts of vitamin A, using sodium amide, triphenylmethyl sodium and potassium *t*-butoxide.

The methyl ether obtained by the action of dimethyl sulfate on the lithium salt was purified by chromatography on activated alumina[11] and was obtained as an orange oil which crystallized from methanol at −70°. The spectrophotometric curve (Fig. 1) of vitamin A methyl ether is identical in all respects with that of vitamin A alcohol, both having absorption maxima at 326 mμ on the Beckman spectrophotometer (model DU). The extinction coefficient ($E^{1\%}_{1\text{ cm.}}$) in isopropanol is 1660, which corresponds to an equivalent extinction coefficient of 1742 for vitamin A alcohol. We found an extinction coefficient of 1769 for crystalline vitamin A alcohol. Crystalline vitamin A methyl ether was found to have a provisional biological potency of 3,500,000 U.S.P. XII units per gram. This value was obtained as the average of three biological assays (a total of 30 rats used) made by Miss Elizabeth Musser of these Laboratories. Baxter and Robeson[12] reported 4,300,000 U. S. P. XI units per gram for crystalline vitamin A alcohol. A value of 3,500,000 for vitamin A alcohol is obtained consistently in our Laboratories.

Since the final step in most of the reported vitamin A ether syntheses involves dehydration, the stability of the ether under these conditions is of particular interest. Although vitamin A methyl ether was found to be more stable than the alcohol toward alcoholic hydrogen chloride by the method of Shantz, *et al.*,[13] absorption spectra studies (Fig. 2) showed that some anhydro vitamin A is formed. Similarly the ether possesses a greater stability than the alcohol toward the action of *p*-toluenesulfonic acid in boiling benzene. For example, at a molar ratio of one part of *p*-toluenesulfonic acid to 218 parts of vitamin A alcohol or its methyl ether, the ether is unaffected, whereas anhydro formation is observed with the alcohol (Fig. 3). However, at a molar ratio of 1:92 anhydro formation is observed with the vitamin A ether, whereas vitamin A acetate is little affected (Fig. 4).

The biological utilization of vitamin A methyl

(7) Cawley, British Patent 578,449 (August 2, 1946).
(8) Gilman, Langham and Moore, THIS JOURNAL, **62**, 2327 (1940).
(9) Distillation Products, Inc., Rochester, New York.
(10) Milas, U. S. Patent 2,296,291 (September 22, 1942).
(11) Grade F20, 80–200 mesh, Aluminum Ore Co., East St. Louis, Illinois.
(12) Baxter and Robeson, THIS JOURNAL, **64**, 2411 (1942).
(13) Shantz, Cawley and Embree, *ibid.*, **65**, 901 (1943).

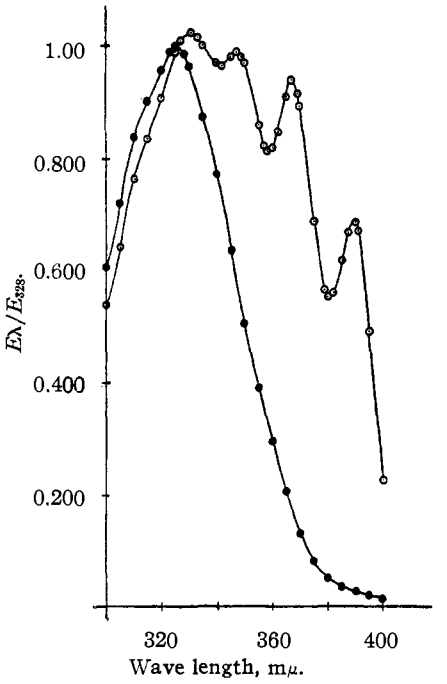


Fig. 3.—Spectrophotometric curves in isopropanol of vitamin A methyl ether, ●, and of vitamin A alcohol, ⊙, after treatment with 0.0046 mole of *p*-toluenesulfonic acid.

ether in the rat is now being investigated and will be reported at a later date.

Experimental

Crystalline Vitamin A Methyl Ether[14]

Crystalline vitamin A alcohol (500 mg., 1.75 millimoles) was dissolved in 60 cc. of dry benzene and the solution further dried by distillation of 10 cc. of benzene. To the solution, at room temperature, was added 6.0 cc. of 0.38 *N* *n*-butyllithium (2.28 millimoles)[8] in low boiling petroleum ether (30–40°). A sharp color change from yellow to deep cherry red occurred immediately. The solution was shaken and allowed to stand at room temperature for five minutes. Then dimethyl sulfate (560 mg., 4.52 millimoles) in 20 cc. of dry benzene was added, followed by a return of the yellow color. The solution was heated at 70° for one hour, cooled in ice, transferred to an amber separatory funnel where it was washed successively with cold solutions of 0.5 *N* sulfuric acid, 1 *N* ammonium hydroxide (twice) and water (three times). The solution was dried over anhydrous sodium sulfate, filtered and concentrated at 40° under reduced pressure to give an orange oil. The last traces of benzene were removed from the residual oil by the repeated addition of purified Skelly Solve B[15] followed by its removal under reduced pressure. An antimony trichloride blue color assay on the above oil showed a 92.5% recovery.

The orange oil was dissolved in 25 cc. of purified Skelly Solve B and put on a 15 × 5 cm. activated alumina column.[11] All reaction products were absorbed at the top of the column. After washing well with Skelly Solve B, the yellow vitamin A methyl ether was eluted with 1% acetone in Skelly Solve B. This gave a sharp separation from an orange pigment which remained at the top of the column. An antimony trichloride blue color assay on the ether fraction showed an over-all yield of 90%.

The oil, obtained on removal of the solvent at 40° under reduced pressure, crystallized from methanol after standing several months at −70°; m. p. 31–33°. Three recrystallizations from methanol and two from Skelly Solve B gave yellow crystals of vitamin A methyl ether; m. p. 33–34°. The yield of crystalline ether from 1 g. of alcohol was 660 mg. (62.7%).

Anal. Calcd. for $C_{21}H_{32}O$: C, 83.95; H, 10.74; OCH_3, 10.34. Found: C, 83.96, 83.76; H, 11.16, 11.07; OCH_3, 10.00, 9.94. The antimony trichloride blue color assay was 100% of the expected value.

Crystalline vitamin A methyl ether is soluble in the common organic solvents such as alcohol, ether, benzene, etc., and is insoluble in water. It has been successfully crystallized from methyl alcohol, ethyl formate and Skelly Solve B, the former being the preferred solvent. For best crystallization results the oil obtained upon chromatography of a 1-g. run is dissolved in 5 cc. of absolute methanol, the solution cooled in ice, seeded, and allowed to stand in a refrigerator overnight, whereupon large crystals are formed. The mixture is then cooled in an ice–salt mixture for four hours, and then at about −70° (solid carbon dioxide) twenty hours. The crystals are filtered on a sintered glass funnel which has been cooled with Dry Ice. Recrystallizations are carried out in the same manner.

Behavior of Vitamin A Methyl Ether in $^1/_{30}$ *N* Dry Hydrogen Chloride in Alcohol (Fig. 2).—Crystalline vitamin A methyl ether (11.5 mg.) was dissolved in 0.75 cc. of absolute alcohol, after which 0.25 cc. of 0.132 *N* dry hydrogen chloride in absolute ethanol was added. The solution was shaken and allowed to stand at room temperature for fifteen minutes. Water (5 cc.) was added and the mixture extracted twice with 10-cc. portions of benzene. The combined benzene solutions were washed once with cold saturated sodium bicarbonate solution and three times with cold water, dried over anhydrous sodium sulfate, filtered and the solvent removed at 40° under reduced pressure to give an oil. The last traces of benzene were removed by repeatedly adding isopropanol followed by its removal under reduced pressure. The spectrophotometric curve was determined on this oil in isopropanol.

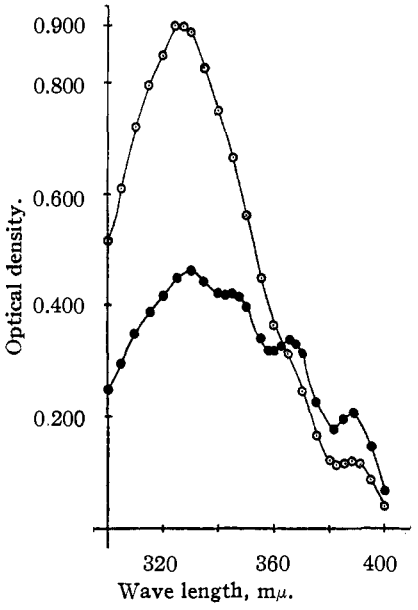


Fig. 4.—Spectrophotometric curves in isopropanol of vitamin A methyl ether, ●, and of vitamin A acetate, ⊙, after treatment with 0.011 mole of *p*-toluenesulfonic acid.

(14) All experiments were carried out in an atmosphere of dry nitrogen in subdued light.

(15) Purified by treatment with concentrated sulfuric acid, neutralization and distillation.

Vitamin A alcohol (10.5 mg.) was treated in exactly the same manner.

Behavior of Vitamin A Methyl Ether with *p*-Toluenesulfonic acid

1. **In a Molar Ratio of 218 to 1 (Fig. 3).**—A solution of 26 micrograms of *p*-toluenesulfonic acid monohydrate in 20 cc. of benzene was dried by distillation of 10 cc. of benzene. To this solution crystalline vitamin A methyl ether (8.9 mg.) was added and the solution refluxed for thirty minutes after which it was cooled in ice, and then poured onto cold saturated sodium bicarbonate contained in a separatory funnel. The benzene solution was washed well with the bicarbonate solution and then three times with cold water, dried over anhydrous sodium sulfate, filtered and concentrated. After the last traces of benzene were removed as above, an ultraviolet absorption curve was run on the residual oil.

Crystalline vitamin A alcohol (8.5 mg.) was run under identical conditions.

2. **In a Molar Ratio of 92 to 1 (Fig. 4).**—Vitamin A methyl ether (9.4 mg.) was treated with 65 micrograms of *p*-toluenesulfonic acid monohydrate exactly as in (1) above. Definite anhydro formation is indicated in the spectrophotometric curve.

Vitamin A acetate (10.6 mg.) under identical conditions was little affected.

Summary

1. Crystalline vitamin A methyl ether has been prepared by the action of dimethyl sulfate on the lithium salt of the alcohol.

2. Crystalline vitamin A methyl ether has a provisional biological potency of 3,500,000 U. S. P. XII units per gram.

3. The behavior of vitamin A methyl ether under dehydration conditions has been studied and a comparison is made between the stability of the vitamin A alcohol, ether and acetate under these conditions.

KALAMAZOO 99, MICHIGAN RECEIVED JUNE 23, 1947

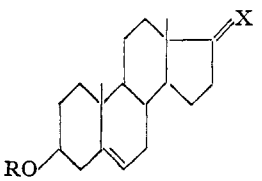
[CONTRIBUTION FROM THE RESEARCH DEPARTMENT OF SYNTEX S. A.]

Mercaptols of 17-Keto Steroids

BY L. NORYMBERSKA, J. NORYMBERSKI AND A. OLALDE

In a recent paper by Hauptmann[1] the reaction of estrone acetate with ethanedithiol was reported. Since the reactions of 17-keto steroids with mercaptans have been the object of investigation in our laboratory for a considerable period of time, we wish to communicate some of the results obtained.[2]

By condensation of dehydroisoandrosterone acetate (II) with benzyl and ethyl mercaptan in the presence of fused zinc chloride and anhydrous sodium sulfate, we were able to prepare the respective mercaptols III and V.



I. R = H, X = O
II. R = CH_3CO, X = O
III. R = CH_3CO, X = $(C_6H_5CH_2S)_2$
IV. R = H, X = $(C_6H_5CH_2S)_2$
V. R = CH_3CO, X = $(C_2H_5S)_2$
VI. R = H, X = $(C_2H_5S)_2$
VII. R = CH_3CO, X = H_2
VIII. R = H, X = H_2

Acid hydrolysis of dehydroisoandrosterone acetate dibenzylmercaptol (III) led to the formation of dehydroisoandrosterone (I); alkaline hydrolysis to dehydroisoandrosterone dibenzylmercaptol (IV); boiling with cadmium carbonate and mercuric chloride in acetone or acetic acid solution to dehydroisoandrosterone acetate (II); hydrogenolysis with Raney nickel to desoxo-dehydroisoandrosterone acetate (VII).

The last-mentioned reaction is of some special interest: Butenandt and Surányi[3] and recently Heard and McKay[4] described the Wolff–Kishner reduction of dehydroisoandrosterone semicarbazone and the product obtained has been proved to consist of a hard to separate mixture of desoxo-dehydroisoandrosterone, etiocholan-3(α)-ol and androstan-3(β)-ol. On the other hand, by hydrogenolysis of the dibenzylmercaptol, as well as of the diethylmercaptol of dehydroisoandrosterone acetate (III and V, respectively) with Raney nickel, we have obtained the pure desoxo-dehydroisoandrosterone acetate (VII) in excellent yield and by its saponification the desoxo-dehydroisoandrosterone (VIII).

Acknowledgment.—The microanalyses have been carried out by L. N. at the microanalytical laboratory of the Escuela Nacional de Ciencias Biológicas, Instituto Politecnico Nacional, with the kind permission of Dr. P. Hope, to whom we wish to express our thanks for his obligingness. Dr. G. Rosenkranz of our laboratory we thank for his kind interest in this work.

Experimental

Dibenzylmercaptol of Dehydroisoandrosterone Acetate (III).—A mixture of 15 g. of dehydroisoandrosterone acetate, 15 cc. of benzylmercaptan, 10 cc. of dioxane and 15 g. of anhydrous sodium sulfate is ice-cooled and 15 g. of freshly fused and pulverized zinc chloride is added.

(1) Hauptmann, THIS JOURNAL, **69**, 562 (1947).

(2) Further communications on this subject will be reported soon.

(3) Butenandt and Surányi, *Ber.*, **75**, 591 (1942).

(4) Heard and McKay, *J. Biol. Chem.*, **165**, 677 (1946).