these were new hydrogenation products of lignin. Data on optimum conditions of catalyst, solvent, and kind of lignin are recorded.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY AND THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE COLLEGE OF AGRICULTURE AND APPLIED SCIENCE]

The Isolation of β-Amyrin from the Leaves and Seeds of Alfalfa

BY L. CARROLL KING, CHARLES D. BALL, BYRON RIEGEL, CARL E. SCHWEITZER, PERRIN G. SMITH AND EDWIN W. MEYER

In the systematic investigation of the components of the unsaponifiable fractions from alfalfa leaf and seed oils, the occurrence of α-spinasterol and carnaubyl alcohol in the leaf meal oil,[1] and of α, β and δ-spinasterols in the seed oil[2] has been reported. In addition to these alcohols, β-amyrin has been isolated[3] from both sources. It was obtained from alfalfa leaf meal oil as the allophanate or as the acetate during the fractionation of that oil for vitamin K_1. It was also isolated from the unsaponifiable fraction of alfalfa seed oil as the acetate after removal of most of the sterols.

Hydrolysis of the acetate or allophanate gave the free alcohol, $C_{30}H_{50}O$. This was converted to the benzoate and to the *p*-nitrobenzoate. The identity of this substance from these alfalfa sources with β-amyrin is indicated by the agreement of the melting points and specific rotations of the alcohol and several of its derivatives, as recorded in Table I, with reported values. β-Amyrin and its derivatives from the two alfalfa sources were directly compared by melting points and mixed melting points.

TABLE I

Source	Elemi gum[a]		Alfalfa seeds		Alfalfa leaves	
Substance	M. p., °C.	$[\alpha]_D$	M. p., °C.	$[\alpha]_D$	M. p., °C.	$[\alpha]_D$
β-Amyrin	192–194	89.1	196–198	86.5	199–200	86.1
Acetate	238–239	82.0	238–239	79.8	238–240	85.1
Benzoate	231–232		233–234	100.1	232–233.4	98.4
p-Nitrobenzoate	257–258	93.2	257–258	95.6	257–258	

[a] J. L. Powers and W. E. Powers, *J. Am. Pharm. Assoc.*, 29, 175 (1940).

The authors from Northwestern University wish to express their appreciation to the Abbott Laboratories for their generous support of this work.

(1) B. Riegel, C. E. Schweitzer and P. G. Smith, *J. Biol. Chem.*, **129**, 495 (1939).

(2) L. C. King and C. D. Ball, THIS JOURNAL, **61**, 2910 (1939); **64**, 2488 (1942).

(3) The study of the constituents of alfalfa seeds was carried out at Michigan State College (L. C. K. and C. D. B.) and that on the leaves at Northwestern University. The discovery that β-amyrin occurs in both sources is due to Dr. King, who is now located at Northwestern University.—B. R.

Experimental[4]

Isolation from Alfalfa Seeds.—From ground alfalfa seeds (Hardigan) was obtained a 11.8% yield of oil[2] which gave about 4% of unsaponifiable material. Fractional crystallization of 150 g. of this material from moist ether separated it roughly into three parts: a solid fraction melting above 120°, a second solid fraction melting below 120° and an oily residue. The higher melting fraction has been previously described.[2] The material melting below 120° (weight 4 g.) was fractionally crystallized from 95% ethanol. By this method more material melting above 120° was removed, leaving a fraction that melted over a range of 30–115°. This fraction was dissolved in 500 ml. of warm acetic anhydride and allowed to stand overnight. The solid material which separated was removed by filtration and dissolved in 200 ml. of boiling acetone. On cooling a flocculent precipitate formed which was removed. Further crystallization from acetone gave waxy material melting at 64–66°. It was insoluble in cold concd. sulfuric acid and appeared to be a hydrocarbon. The acetone filtrates were concentrated to 100 ml. and allowed to stand. Large rod-like and large flaky crystals slowly separated along with more of the flocculent precipitate. The mixture was filtered in such a way as to retain only the large rod-like and flaky crystals. These were then separated manually. The flaky material consisted mostly of β-spinasteryl acetate. The rod-like material was recrystallized from acetone to give 0.5 g. of beautiful crystals melting at 238–239°; $[\alpha]^{19}D$ +79.8° (50.6 mg. made up to 2 ml. with chloroform, $\alpha^{19}D$ +4.04°; *l*, 2 dm.). The crystals were identified as β-amyrin acetate.

Anal. Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.83, 81.79, 81.97; H, 11.41, 11.05, 11.20.

β-Amyrin.—A portion of the above acetate was saponified with 5% alcoholic potassium hydroxide. The product was crystallized from 95% ethanol giving material that melted at 195–198°; $[\alpha]^{18}D$ +86.5° (24.8 mg. made up to 2 ml. with chloroform, $\alpha^{18}D$ +2.15°; *l*, 2 dm.).

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.55, 84.52; H, 11.41, 11.85.

This alcohol on heating with acetic anhydride gave in almost quantitative yield, a product identical with the original acetate; m. p. 234–238°.

β-Amyrin Benzoate.—A solution of 50 mg. of the above β-amyrin in 1 ml. of pyridine was treated with about 0.5 ml. of benzoyl chloride. The reaction mixture was heated for three hours on a steam-bath and the product recovered

(4) All melting points are corrected.

in the usual manner. Crystallization from acetone gave a pure product melting at 233–234°; $[\alpha]^{24}D$ +101.10 (37 mg. made up to 2 ml. with chloroform, $\alpha^{24}D$ +1.87°; *l*, 1 dm.).

β-Amyrin *p*-Nitrobenzoate.—To a solution of 30 mg. of the above β-amyrin in 3 ml. of pyridine was added 200 mg. of *p*-nitrobenzoyl chloride. After heating for three hours on a steam-bath the product was recovered and crystallized from a large volume of 95% ethanol; yield 15 mg.; m. p. 257–258°; $[\alpha]^{25}D$ +95.6° (13.8 mg. made up to 2 ml. with chloroform, $\alpha^{25}D$ +0.66°; *l*, 1 dm.).

Isolation from Alfalfa Leaves (a) as the Allophanate.—α-Spinasterol, carnaubyl alcohol, β-amyrin and a pentacosane were isolated while fractionating the oil from alfalfa leaves[1] for vitamin K_1. During numerous enrichment operations, β-amyrin persistently accompanied the vitamin. Dehydrated alfalfa leaf meal was exhaustively extracted with petroleum ether. After completely removing the solvent the dark viscous residue was molecularly distilled. The fraction collected between 120–150° at 10^{-3} mm. contained most of the unsaponifiable material.[1] This fraction contained 20–22% of phytosterols, most of which could be removed by crystallization from an acetone solution. The acetone was then completely removed from the filtrate, and the dark red residue was dissolved in petroleum ether. Treatment of this solution with small amounts of activated Florex removed all but traces of the remaining phytosterols. The sterol-free residue, after evaporation of the petroleum ether, was again subjected to molecular distillation and the fraction distilling at 115–140° at 10^{-3} mm. was collected. A petroleum ether solution of this fraction was treated with activated Florex which adsorbed several components including the vitamin K_1 and β-amyrin. Elution with petroleum ether containing 5% methanol gave a 4% vitamin K_1 concentrate after removing the Florex and solvent. On cooling an acetone solution of this concentrate in a dry-ice bath about one-half of the inert material was precipitated. The insoluble material was reprecipitated several times to remove the vitamin K activity. Concentration of the acetone soluble fraction gave material containing about 8% vitamin K_1. At the time this work was being carried out, vitamin K_1 was shown to be a quinone. An attempt was made to isolate the vitamin as the allophanate of the hydroquinone. An acetone solution of 5.0 g. of the 8% concentrate was treated with an alcohol solution of digitonin to precipitate the last traces of sterols. The filtrate was diluted with ether to remove the excess digitonin. Evaporation of the solvent left 4.56 g. of sterol-free oil. A solution of 4.27 g. of this oil in 95% alcohol was treated with a solution of titanium trichloride to reduce the vitamin K_1. Freshly-boiled cooled water and peroxide-free ether were added. The ether layer was washed two times with air-free water to remove the inorganic material. Benzene was added to the ether solution and the solvents removed. The residue was dissolved in 300 ml. of benzene and 100 ml. was distilled to remove the last traces of water. Cyanic acid gas, obtained from the decomposition of 10 g. of cyanuric acid in a stream of nitrogen, was passed into the cooled benzene solution. The flask was tightly stoppered and allowed to stand in the refrigerator for a week. An insoluble residue, cyamelide, formed which was removed by filtration. Concentration of the filtrate gave more insoluble material as an orange slush. This was separated by filtration and washed with acetone, giving a white powder melting at 262–265°. The cyamelide residue was exhaustively extracted with chloroform which on cooling gave more of the white powder melting at 270–274°. This amorphous material was combined and crystallized from a mixture of dioxane and methyl ethyl ketone giving 0.5 g. of very small needles melting at 263–265° (with dec.).

Anal. Calcd. for $C_{32}H_{52}O_3N_2$: N, 5.47. Found: N, 5.58, 5.69.

The allophanate was hydrolyzed and the product converted to the acetate which melted at 238–240° and was identical with the acetate described below.

(b) As the Acetate.—An attempt was made to isolate vitamin K_1 as the diacetate of its hydroquinone from the above described 8% concentrate. To a boiling solution of 6 g. of the 8% K_1 concentrate and 0.3 g. of sodium acetate in 15 ml. of glacial acetic acid and 60 ml. of acetic anhydride was added, in small portions, 7 g. of zinc dust. The dark red solution turned a light yellow. The heating was continued for about one hour and then the excess acetic anhydride was decomposed with methanol. The mixture was poured into water and extracted with ether. The ether solution was washed with water, dilute sodium carbonate, dried and the ether removed. A bright orange oil remained which became filled with crystals on cooling. Two crops were removed melting at 234–235° and 237–238°, but the amount obtained was too small for further work. A larger run was made on 35 g. of the above described 4% K_1 concentrate. It was first reductively acetylated, and then subjected to low temperature precipitation. The acetone insoluble fraction which gave no K activity amounted to about 21 g. The soluble fraction, after removing the acetone, slowly deposited crystals. After standing one and a half years they were removed by filtration and washed with a small amount of acetone, yield 2.2 g.; m. p. 225–257°. Crystallization from methyl ethyl ketone gave 1.15 g. of beautiful brilliant prisms melting at 238–239°. Another crystallization from ethyl acetate raised the m. p. to 238–240°; $[\alpha]^{25}D$ + 85.1° (39.2 mg. made up to 2.42 ml. with chloroform, $\alpha^{25}D$ + 1.38°, *l*, 1 dm.). These crystals gave no vitamin K activity and were obtained from the K_1 concentrates even when the last traces of sterols were removed by digitonin. When the crystals were mixed with the corresponding crystals from alfalfa seeds they gave no depression in melting point.

Anal. Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.61, 81.65; H, 10.68, 10.83.

β-Amyrin.—Saponification of the above acetate with alcoholic potassium hydroxide gave crystals melting at 194–195°. Recrystallization from acetone or ethyl acetate gave small needles melting at 199–200°; $[\alpha]^{23}D$ + 86.1° (30.2 mg. made up to 2 ml. with chloroform, $\alpha^{23}D$ + 1.30°; *l*, 1 dm.). This product gave no mixed melting point depression with the alcohol isolated from alfalfa seed oil.

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.26, 84.29; H, 11.34, 11.78.

β-Amyrin Benzoate.—A 95-mg. sample of the alcohol was converted to the benzoate in a manner similar to that described above. Crystallization from acetone gave small plates melting at 232–233.4°; $[\alpha]^{22.5}D$ + 98.4° (15.3 mg. made up to 2 ml. with chloroform, $\alpha^{22.5}D$ + 0.753°; *l*, 1

dm.). When mixed with the corresponding derivative from alfalfa seed oil, the melting point was not depressed.

Anal. Calcd. for $C_{37}H_{52}O_2$: C, 83.72; H, 10.25. Found: C, 83.73; H, 9.96.

β-Amyrin *p*-Nitrobenzoate.—A small sample of the β-amyrin from alfalfa leaves was converted to the *p*-nitrobenzoate in a manner similar to that previously described. The product when crystallized twice from ethanol gave a m. p. of 257–258°. This product gave no depression in m. p. when mixed with the previously described *p*-nitrobenzoate.

Summary

1. β-Amyrin has been isolated from the unsaponifiable fraction of alfalfa seed oil (Hardigan).
2. It has also been isolated from alfalfa leaf meal oil.
3. β-Amyrin from these sources has been characterized by its acetate, benzoate and *p*-nitrobenzoate.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, McGILL UNIVERSITY]

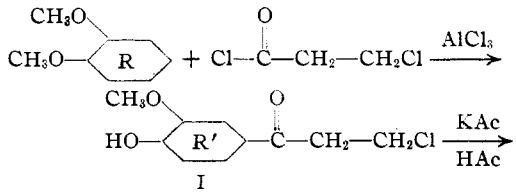
Studies on Lignin and Related Compounds. LXIV. Synthesis and Properties of 3-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone[1]

BY KENNETH A. WEST[2] AND HAROLD HIBBERT

The relationship between certain oxygenated propyl phenol derivatives and lignin and their possible role as plant respiratory catalysts have been discussed recently by Hibbert.[3]

In a previous paper[4] the synthesis of 3-hydroxy-1-(3,4-dimethoxyphenyl)-1-propanone[5] was described and the possibility of the existence in wood of a lignin progenitor of the type R—CO—CH_2—CH_2OH discussed. It was considered[3] possible that its derivative (R—CHOH—CH—CH_2OH) may play the same role in the plant respiratory system as is performed by citric (or isocitric) acid in the animal system (Krebs). The present communication deals with the synthesis of the unmethylated derivative, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (III), and a study of its properties, in particular its behavior toward common lignin extractants.

The synthesis was carried out according to the following series of reactions



I

$$R'—CO—CH_2—CH_2OAc \xrightarrow[H_2O]{BaCO_3} R'—CO—CH_2—CH_2OH$$

II III

The synthesis of the chloride (I) is dependent upon simultaneous condensation and demethylation reactions similar to those recorded in the literature in the syntheses of 4-hydroxy-3-methoxy-α-chloroacetophenone[6] and 2-bromo-1-(4-hydroxy-3-methoxyphenyl)-1-propanone[7] obtained by the condensation of veratrole with α-bromoacetyl bromide and α-bromopropionyl bromide, respectively. Each of these reactions takes place at room temperature in the presence of two equivalents of aluminum chloride whereas under the same conditions veratrole and β-chloropropionyl chloride condense to give a 75% yield of 3-chloro-1-(3,4-dimethoxyphenyl)-1-propanone, no appreciable demethylation occurring. 3-Chloro-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (I) was finally synthesized by heating a mixture of veratrole and β-chloropropionyl chloride in the presence of *four* equivalents of aluminum chloride at 50° for four hours followed by twenty minutes at 100°; yield after two recrystallizations, 60%. Each product in this series of reactions was methylated with diazomethane and the methylated derivative compared with the corresponding member of the veratryl series.[4]

It was necessary to show that demethylation had taken place in the position *para* and not *meta* to the propyl side chain. This was done by ethylating the acetate (II) with diethyl sulfate and alkali and then subjecting the ethyl ether to per-

(1) From a thesis submitted to the Graduate Faculty of McGill University by Kenneth A. West in partial fulfilment of the requirements for the degree of Doctor of Philosophy, May, 1942.

(2) Holder of a National Research Council of Canada Studentship, 1941–1942.

(3) Hibbert, *Ann. Rev. Biochem.*, **11**, 183 (1942).

(4) West, Hawkins and Hibbert, THIS JOURNAL, **63**, 3035 (1941).

(5) The question of correct nomenclature for the numerous new derivatives arising from these researches has occasioned the senior author (H. H.) much concern. After consultation with Dr. Austin Patterson it seems advisable, in the future, to follow closely the Geneva system to promote clarity and avoid confusion.

(6) Pratt and Robinson, *J. Chem. Soc.*, **123**, 245 (1923).

(7) Cramer and Hibbert, THIS JOURNAL, **61**, 2204 (1939).