

- (8) Harries, H., and Himmelmann, O., *Ber.*, 41 (1908), 2187.  
(9) Pickard, R. J., *et al.*, *J. Chem. Soc.*, 117 (1920), 1248.  
(10) Kuwata, T., *J. Soc. Chem. Ind., Japan*, 34 (1931), 70B.

## The Isolation and Identification of Alpha- and Beta-Amyrin from the Bark of *Viburnum Opulus*\*

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In the course of a chemical examination of the bark of *Viburnum opulus*,<sup>1</sup> a considerable quantity of a wax-like material soluble in petroleum ether (b. p. 60–75°) was obtained. This, upon saponification and subsequent removal of the soap, yielded a yellowish colored unsaponifiable portion amounting to nearly 50% of the weight of the wax. The unsaponifiable portion, after several recrystallizations from alcohol, with the aid of charcoal, became nearly colorless, but did not possess a reasonably sharp melting point. This material produced the characteristic color reactions of sterols (1), (2), (3), (4). However, the quantity obtained seemed to preclude the possibility of its consisting entirely of a mixture of sterols.

Attempts to make a separation of the material through fractional crystallization of the acetates or benzoates were unsuccessful. Finally digitonin was used to precipitate all sterols precipitable by that reagent. The fraction which was not precipitated by the digitonin, as was expected, constituted the major portion of the mixture. It now melted at 165–172°, even after many recrystallizations from various solvents. After several attempts to make a further separation, it was found that after benzoylating the mixture a separation could be made by means of low-boiling petroleum ether (b. p. 30–40°). Two benzoates, one melting at

194–195° and constituting the major portion, and the other melting at 230–232° and constituting the minor portion, were obtained. The first benzoate upon hydrolysis yielded an alcohol melting at 182–184°; the second, an alcohol melting at 191–193°. Carbon and hydrogen determinations upon the alcohols and upon the benzoates indicated that probably both alcohols possessed the formula  $C_{30}H_{50}O$ . It seemed reasonable to expect that these might be known compounds, and it was found that the physical and chemical properties of the first isomer were in fairly close agreement with those given in the literature for  $\alpha$ -amyrin, while the properties of the second were in close agreement with those given for  $\beta$ -amyrin. Moreover, it was found that apparently the best method of separation of the mixed amyryns was that described by Horrmann and Firzlaff (5), through the benzoates by means of pentane.

In order to test the correctness of the assumption that both  $\alpha$ - and  $\beta$ -amyrin were present in *Viburnum opulus* bark, these compounds were isolated from Manila Elemi Gum,<sup>2</sup> converted into the corresponding acetates, benzoates and nitrobenzoates, and compared with corresponding derivatives of the alcohols obtained from the bark of *Viburnum opulus*. The alcohols and derivatives of them from both sources were quite similar in physical and chemical properties, and it is believed that *Viburnum opulus* bark undoubtedly contains both amyryns.

The amyryns were first obtained from Elemi Gum by Rose (6), and were first separated into two modifications by Vesterberg (7) through fractional crystallization of the acetates from ligroin. More recent work, relating to the structure of the amyryns, has been done by Ruzika (8), (9), Brunner (10), Dieterle and Salomon (11), Rollett (12) and others (5), (13), (14), (15). Despite the extensive literature on the amyryns, there is little to indicate that they are widely distributed in plants. They have been reported as constituents of *Asclepias syriaca* (16), of the latex from the milk tree, *Bros-*

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<sup>2</sup> Purchased from the S. B. Penick Company.

*miun galactodendron* (17) and of Mulberry bark (18). It therefore seems of some interest that the amyryns have been found in the bark of *Viburnum opulus*.

#### EXPERIMENTAL

*Isolation of Mixed Amyryns from Viburnum Opulus Bark.*<sup>3</sup>—Eight kilograms of the coarsely powdered bark were exhausted by extraction with 95% alcohol in a Lloyd extraction apparatus. The extract was concentrated to a syrup-like consistence weighing 1850 Gm. This concentrate was then extracted several times with 750-cc. portions of petroleum ether (b. p. 60–75°). The petroleum ether extracts were combined, and the solvent was removed by distillation, leaving a residue which weighed 235 Gm. Saponification value, 99.32, 102.5; iodine number 55.11, 56.96.

Two hundred grams of the petroleum ether extract were heated during six hours on a steam bath under a reflux condenser, with 1 L. of an 8% alcoholic solution of potassium hydroxide. The greater part of the alcohol was removed by distillation, and the residue was poured into 500 cc. of water. The aqueous mixture was extracted with several portions of ether. The ethereal solution, after being shaken twice with water, was dried with anhydrous sodium sulfate and then the ether was removed by distillation. The residue, constituting the unsaponifiable portion, was granular in appearance and slightly yellow in color and weighed 94 Gm. After several recrystallizations from alcohol a nearly colorless crystalline material was obtained which began to soften at 125°, but did not completely melt until a temperature of 160° was attained.

Fifty grams of the partially purified unsaponifiable material were dissolved in 1500 cc. of absolute alcohol. To this solution were added 1250 cc. of a 1% solution of digitonin (Merck) in absolute alcohol, the amount of digitonin required having been determined by a previous small-scale experiment. When the mixture was heated on a steam bath, the digitonide of the sterol present precipitated. This was cooled to room temperature and the digitonide removed by filtration. When the filtrate was concentrated to a volume of 1 L., a small additional amount of the digitonide precipitated, and was removed by filtration. Finally the alcoholic filtrate was concentrated to a volume of 200 cc., which upon cooling deposited a crop of crystals. By further concentration, additional crops of crystals were obtained, which when combined and dried at 100° weighed 44 Gm., and melted at 168–172°. Recrystallization from alcohol did

not raise the melting point. Iodine number,<sup>4</sup> 38.8, 38.55.

*Analysis.* Calcd. for  $C_{30}H_{50}O$ : C, 84.5; H, 11.8; Found: C, 84.41, 84.25; H, 12.02, 12.11; mol. wt. calcd., 426.4. Found (Rast): 438, 442.

*Benzoylation of Mixed Amyryns.*—One hundred grams of the material obtained as described above were dissolved in 500 cc. of anhydrous benzene contained in a one-liter round-bottom flask, 40 Gm. of anhydrous pyridine were added, and finally 70 Gm. of benzoyl chloride. The mixture was heated under a reflux condenser during 4 hours on a steam bath. The mixture was cooled to room temperature, and 500 cc. of ether were added. The ethereal solution was extracted with five 100-cc. portions of 5% sulfuric acid, shaken several times with water, and finally the solvent was removed by distillation. The solid residue which remained was removed from the flask, powdered finely and shaken mechanically during 12 hours with one liter of a cold aqueous 10% solution of sodium carbonate. After filtration, the residue was washed with water until freed from sodium carbonate, and then first air dried, and finally dried in an oven at 100°. The mixed benzoates, after one recrystallization from a mixture of equal volumes of acetone and methyl-ethyl ketone, melted at 176–182°. Yield, 108.5 Gm., 87.5% of the calculated.

*$\beta$ -Amyrin Benzoate.*—One hundred grams of the mixed benzoates were shaken mechanically with one liter of cold petroleum ether (b. p. 30–40°) for 1 hour, and the mixture was filtered. The undissolved residue weighing 28 Gm. was then extracted with 3 successive 300-cc. portions of petroleum ether. The material which finally remained undissolved weighed 15 Gm., and melted at 222–226°. After one recrystallization from benzene and one from a mixture of acetone and methyl-ethyl ketone it melted at 231–232°. When mixed with an authentic sample of  $\beta$ -amyrin benzoate there was no depression in melting point.

*Analysis.* Calcd. for  $C_{35}H_{54}O_2$ : C, 83.8; H, 10.2. Found: C, 83.55, 83.7; H, 10.38, 10.30.

*$\alpha$ -Amyrin Benzoate.*—The petroleum ether from the first liter of extract of the mixed amyryns was removed by distillation, leaving a residue weighing 70 Gm. This, after two recrystallizations as described for  $\beta$ -amyrin benzoate, yielded 58 Gm. of  $\alpha$ -amyrin benzoate melting at 191–193°. When mixed with authentic  $\alpha$ -amyrin benzoate there was no depression in melting point.

*Analysis.* Calcd. for  $C_{35}H_{54}O_2$ : C, 83.8; H, 10.2. Found: C, 83.85, 83.72; H, 10.35, 10.42.

*Hydrolysis of  $\alpha$ -Amyrin Benzoate.*—Fifty grams of  $\alpha$ -amyrin benzoate were added to one liter of a 4% alcoholic solution of potassium hydroxide. The mixture was heated under a reflux condenser during 12 hours on a steam bath, most of the alcohol was removed by distillation, and water was added,

<sup>3</sup> The *Viburnum opulus* bark used in this investigation was purchased from the S. B. Penick Company. It was identified as authentic by Professor Heber Youngken, Massachusetts College of Pharmacy, and by Professor C. C. Glover, College of Pharmacy, University of Michigan.

<sup>4</sup> This determination was made by Mr. Kenneth Latimer.

and the mixture was filtered. The residue was washed several times with water, and upon drying it weighed 40 Gm. After several recrystallizations from ethyl acetate, it melted at 182–184°. When mixed with authentic  $\alpha$ -amyrin there was no depression in melting point.

*Analysis.* Calcd. for  $C_{30}H_{50}O$ : C, 84.5, H, 11.8. Found: C, 84.28, 84.35; H, 12.05, 11.95.

For  $\alpha$ -amyrin from *Viburnum opulus*:  $[\alpha]_D^{30} = +84^\circ$  (Chloroform).

For  $\alpha$ -amyrin from Elemi Gum:  $[\alpha]_D^{30} = +84^\circ$  (Chloroform).

*Hydrolysis of  $\beta$ -Amyrin Benzoate.*—Eight grams of  $\beta$ -amyrin benzoate were hydrolyzed in the same manner as described for  $\alpha$ -amyrin benzoate. The product, recrystallized several times from ethyl acetate, melted at 192–194°. When mixed with authentic  $\beta$ -amyrin there was no depression in melting point. Yield, 6.2 Gm.

*Analysis.* Calcd. for  $C_{30}H_{50}O$ : C, 84.5; H, 11.8. Found: C, 84.32, 84.27; H, 11.85, 11.92.

For  $\beta$ -amyrin from *Viburnum opulus*:  $[\alpha]_D^{30} = +89.2^\circ$  (Chloroform).

For  $\beta$ -amyrin from Elemi Gum:  $[\alpha]_D^{30} = +89.1^\circ$  (Chloroform).

*Preparation of  $\alpha$ -Amyrin Acetate.*—Two grams of  $\alpha$ -amyrin were acetylated by means of acetic anhydride. Yield, 1.8 Gm. After one recrystallization from a mixture of acetone and methyl-ethyl ketone it melted at 224–225°. When mixed with authentic  $\alpha$ -amyrin acetate there was no depression in melting point.

For  $\alpha$ -amyrin acetate derived from *Viburnum opulus* amyrin:  $[\alpha]_D^{30} = +74.4^\circ$  (Chloroform).

For  $\alpha$ -amyrin acetate derived from Elemi Gum:  $[\alpha]_D^{30} = +74.4^\circ$  (Chloroform).

*Preparation of  $\beta$ -Amyrin Acetate.*—Acetylated  $\beta$ -amyrin, recrystallized from the same solvent as  $\alpha$ -amyrin acetate, melted at 238–239°. When mixed with authentic  $\beta$ -amyrin acetate there was no depression in melting point. Yield, 2 Gm.

For  $\beta$ -amyrin acetate derived from *Viburnum opulus* amyrin:  $[\alpha]_D^{30} = +82^\circ$  (Chloroform).

For  $\beta$ -amyrin acetate derived from Elemi Gum:  $[\alpha]_D^{30} = +81.9^\circ$  (Chloroform).

*Preparation of  $\alpha$ -Amyrin-*p*-nitrobenzoate.*—Two grams of  $\alpha$ -amyrin were added to a solution of 2 Gm. of *p*-nitrobenzoyl chloride in 20 cc. of anhydrous pyridine, and the mixture was heated under a reflux condenser during 3 hours, then cooled and poured into 150 cc. of a 10% aqueous solution of sulfuric acid to which ice had been added. By means of ether, the ester was extracted, and after removal of the solvent, shaken vigorously with a 10% solution of sodium carbonate. After filtration, the nitrobenzoate was washed with water until free of sodium carbonate, air dried, and then dried in an oven at a temperature of 110°. After two recrystallizations from a mixture of acetone and methyl-ethyl ketone, the product weighed 2.15 Gm., 80% of the calculated amount. Melting point,

205–206°. When mixed with an authentic sample of  $\alpha$ -amyrin-*p*-nitrobenzoate, there was no depression in melting point.

For  $\alpha$ -amyrin-*p*-nitrobenzoate from *Viburnum opulus*  $\alpha$ -amyrin:  $[\alpha]_D^{30} = +87.3^\circ$  (Chloroform).

For authentic  $\alpha$ -amyrin-*p*-nitrobenzoate:  $[\alpha]_D^{30} = +87.2^\circ$  (Chloroform).

*Preparation of  $\beta$ -Amyrin-*p*-nitrobenzoate.*— $\beta$ -Amyrin was esterified by a method identical with that described for  $\alpha$ -amyrin. Recrystallized in the same manner as  $\alpha$ -amyrin-*p*-nitrobenzoate, the product melted at 257–258°. When mixed with authentic  $\beta$ -amyrin-*p*-nitrobenzoate there was no depression in melting point.

For  $\beta$ -amyrin-*p*-nitrobenzoate from *Viburnum opulus* amyrin:  $[\alpha]_D^{30} = +93.2^\circ$  (Chloroform).

For authentic  $\beta$ -amyrin-*p*-nitrobenzoate:  $[\alpha]_D^{30} = +93.1^\circ$  (Chloroform).

*Isolation of Mixed Amyrins from Manila Elemi Gum.*—One kilogram of Manila Elemi Gum was shaken mechanically during 5 hours with one liter of alcohol. The mixture was filtered, and the undissolved amyryns thus obtained were recrystallized once from alcohol. Yield, 180 Gm. Melting point, 165–171°.

*Separation of Mixed Amyrins from Elemi Gum.*—The mixed amyryns were benzoylated and the benzoates were separated into  $\alpha$ - and  $\beta$ -modifications by means of petroleum ether (b. p. 30–40°), as described for amyryns from *Viburnum opulus* bark. The benzoates were hydrolyzed and the products purified by the same methods as those described, and with comparable results.

*Preparation of Esters of Manila Elemi Amyrins.*—Acetates and *p*-nitrobenzoates of both  $\alpha$ - and  $\beta$ -amyrin were prepared by the same methods, and with essentially the same results, as those described for the corresponding esters made from *Viburnum opulus* bark amyryns.

#### SUMMARY AND CONCLUSIONS

1. Both  $\alpha$ - and  $\beta$ -amyrin have been isolated from *Viburnum opulus* bark.

2. Both amyryns from *Viburnum opulus* bark have been identified by comparison with authentic amyryns isolated from Manila Elemi Gum.

3. Acetates, benzoates and *p*-nitrobenzoates of both  $\alpha$ -amyrin and  $\beta$ -amyrin obtained from *Viburnum opulus* bark are similar in physical and chemical properties to corresponding esters of authentic  $\alpha$ - and  $\beta$ -amyrin prepared from amyryns obtained from Elemi Gum.

#### REFERENCES

- (1) Salkowski, E., *Z. physiol. Chem.*, 57 (1908), 523.

- (2) Liebermann, C., *Ber.*, 18 (1885), 1805.  
 (3) Burchard, *Chem. Zentr.*, 61, (1) (1890), 25.  
 (4) Rosenheim, O., *Biochem. J.*, 23 (1928), 47.  
 (5) Horrmann, P., and Firzlafl, J., *Arch. Pharm.*, 268 (1930), 64.  
 (6) Rose, H., *Ann.*, 13 (1835), 192.  
 (7) Vesterberg, A., *Ber.*, 20 (1887), 1242; 23 (1890), 3186.  
 (8) Ruzika, L., *Ann.*, 471 (1929), 21.  
 (9) Ruzika, L., *Helv. Chim. Acta*, 15 (1932), 482; *Ibid.*, 1285; 18 (1935), 61.  
 (10) Brunner, O., *Monatsh.*, 50 (1928), 284; 61 (1933), 79; 63 (1933), 79.  
 (11) Dieterle, H., and Salomon, A., *Arch. Pharm.*, 269 (1931), 78; 270 (1932), 495; 271 (1933), 177.  
 (12) Rollett, A., *Monatsh.*, 47 (1926), 437; 58 (1931), 113; 63 (1933), 311.  
 (13) Horrmann, P., and Martinius, J., *Arch. Pharm.*, 272 (1934), 607.  
 (14) Jacobs, W., and Fleck, E., *J. Biol. Chem.*, 88 (1930), 137.  
 (15) Spring, F., *J. Chem. Soc.*, (1933), 1345; (1934), 650.  
 (16) Schmid, L., *Monatsh.*, 47 (1926), 433; 48 (1927), 577.  
 (17) Gomez, J., *Ann. soc. espan. fis.*, 33 (1935), 360; through *Chem. Abstracts*, 29 (1935), 6925.  
 (18) Oku, M., *J. Agr. Chem. Soc., Japan.*, 12 (1936), 595; through *Chem. Abstracts*, 30 (1936), 6786.

## The Oxidation of Salicylates in Alkaline Solutions\*.<sup>†</sup>

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Salicylate solutions, which are colorless when freshly prepared, are rapidly discolored by the formation of a brown oxidation product resulting from the activity of atmospheric oxygen. Numerous past studies of this reaction have been concerned only with the rate of discoloration and the influencing factors (1-13). Reviews of this literature are available in references (6), (8) and (13). It was the purpose of this research to isolate and study the structure of the brown oxidation product obtained from salicylates.

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<sup>†</sup> Abstracted from the dissertation submitted by Dr. Brecht to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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A few oxidation reactions of salicylates have been characterized, but none of the products was similar to that from aged, brown salicylate solutions (14-17). The sodium salt of gentisic acid (2,5-dihydroxybenzoic acid) was obtained by the oxidation of sodium salicylate by magnesium persulfate in alkaline solution (14). Gentisic acid was obtained as a colorless compound. Solutions of gentisic acid rapidly turned brown when exposed to air (15). Gentisic acid was converted by manganese dioxide in sulfuric acid to an unnamed compound of the formula  $C_{14}H_6O_6$ . This compound was obtained as an ochre-yellow crystalline powder from *o*-cresol. It sublimed at 230° to sulfur-yellow needles, and melted above 360° (16). This compound is interesting because it is produced from an oxidation product of a salicylate, but it is a different compound than the brown substance obtained from alkaline solutions by atmospheric oxidation. Gentisic acid may be an intermediate product in all oxidations of salicylates.

For the purpose of reference, until a chemical name can be applied, it is proposed to refer to the brown oxidation product from salicylates, a trisodium salt of an organic radicle, as "sodium salicylate-brown." The acid form will be termed "acid salicylate-brown."

### EXPERIMENTAL

The oxidation of sodium salicylate and other related compounds was studied in three types of media: (a) slightly acidic, (b) slightly alkaline, containing 2.5 per cent each of sodium bicarbonate and sodium carbonate and (c) strongly alkaline, containing 25 per cent of sodium hydroxide. Two oxidizing agents were used: air and hydrogen peroxide.

*Slightly Acidic.*—Atmospheric oxygen: Solutions of sodium salicylate, saturated with salicylic acid, showed no discoloration upon aging.

Hydrogen peroxide: One gram of salicylic acid, or a similar compound, was mixed with 1 cc. of water and 5 cc. of 30 per cent hydrogen peroxide solution in a capacious beaker. No reaction took place until 5 drops of ferric chloride test solution were dropped on different areas of the mixture. The reaction then took place immediately with the evolution of sufficient heat to cause boiling of the water. The reaction was complete in about fifteen seconds. The amount of brown product was deter-