

[CONTRIBUTION FROM THE UNIVERSITY OF MARYLAND]

**Cerin and Friedelin. I. Their Molecular Weights and Empirical Formulas<sup>1</sup>**

BY NATHAN L. DRAKE AND ROBERT P. JACOBSEN

References in the literature to cerin and friedelin, the so-called "cork alcohols," are to be found as far back as 1807, when Chevreul described a material obtained by the extraction of ground cork with alcohol.<sup>2</sup> Chevreul believed that he was dealing with a single substance and named it "cerine" because the product resembled a wax. Further study proved conclusively that cerin was not a wax, but no analyses and no melting point were recorded for the product in this early work. Subsequently several independent researches were conducted on similar material,<sup>3</sup> but the first account of a product which we now know was substantially pure, was that of Thoms,<sup>4</sup> who described the purification of cerin, and gave analytical data which demonstrate that his cerin was pure. Mention is made in Thoms' writing of an acetyl and a benzoyl derivative of cerin, but no details are given. Thoms believed that cerin might be related to the phytosterols, and consequently carried out the usual color tests for this group. His cerin gave a rose red color when treated with acetic anhydride and concentrated sulfuric acid, and a yellow color which became violet after several hours of standing when treated with chloroform and concentrated sulfuric acid. As we shall show later, these color reactions were probably due to impurities of sterol nature.

The important fact that the sparingly soluble crystalline material from cork consisted principally of at least two substances which could be separated by a series of recrystallizations from chloroform, and which differed markedly in both solubility in chloroform and percentage composition was demonstrated by Istrati and Ostrogovich.<sup>5</sup> Friedel,<sup>6</sup> in a note to Istrati, had called the attention of the latter to the fact that Chevreul had previously isolated cerin, adding that he himself had studied the compound and that it appeared to contain a carbonyl group. In honor

of Friedel, Istrati named the more soluble of the two compounds friedelin.

Analyses and molecular weight determinations led Istrati to propose the formula  $C_{27}H_{44}O_2$  for cerin, and either  $C_{21}H_{34}O$ , or  $C_{43}H_{70}O_2$  for friedelin. Both substances in acetic anhydride were found to give a red color with fuming sulfuric acid, but only a faint color with concentrated sulfuric acid; chloroform solutions of each gave no violet color with concentrated sulfuric acid. These findings are in accord with our own work and at variance with the experience of Thoms.<sup>4</sup>

Recently, Winterstein<sup>7</sup> has classified cerin among the sapogenins as a dihydroxy triterpenoid. The literature contains no record of investigation of the structure of cerin or friedelin.

From early work in this Laboratory it became apparent that previous investigators, with the possible exception of Thoms, had not dealt with homogeneous material. It became essential, therefore, to study carefully the purification of cerin and friedelin, preliminary to establishing their percentage compositions and molecular weights. The cerin and friedelin used in this work were obtained by extracting ground cork<sup>8</sup> with ethyl acetate. The crude mixture of cerin and friedelin thus obtained can be separated fairly effectively by taking advantage of the fact that cerin is rather sparingly soluble in chloroform whereas friedelin is very soluble. By fractional crystallization from chloroform, and finally from benzene, pure cerin can be obtained. Cerin so prepared crystallizes from chloroform or benzene in long silky laths which melt with apparent decomposition at 247–251°. The amount of cerin obtainable varies from 5 to 10% of the total weight of the crude material subjected to fractionation.

The friedelin which makes up the bulk of the crude product, together with brown colored matter, remains dissolved in the chloroform filtrates from the above process. By concentrating these until solid begins to separate from the hot solution, and then adding an equal volume of acetone,

(1) From a thesis submitted to the Graduate School in partial fulfillment of the requirements for the Ph.D. degree.

(2) Chevreul, *Ann. chim.*, **62**, 323 (1807); **96**, 141 (1815).

(3) For a discussion of this work see R. P. Jacobsen, "The Isolation of Friedelin and Cerin from Cork and a Study of the Properties and Molecular Weight of Friedelin," Thesis, University of Maryland, 1935.

(4) Thoms, *Pharm. Zentralhalle*, **39**, 699 (1898).

(5) Istrati and Ostrogovich, *Compt. rend.*, **128**, 1581 (1899).

(6) Friedel, *Bull. soc. chim.*, [31] **7**, 164 (1892).

(7) Winterstein, *et al.*, *Z. physiol. Chem.*, **202**, 207 (1931).

(8) Thanks are due the Armstrong Cork and Linoleum Company for supplying the cork used.

friedelin may be precipitated. The small quantity of brown color which remains can be removed by leaching the product with hot acetone in which friedelin is only very sparingly soluble. Pure friedelin can be obtained from this "friedelin rich" material by conversion to an ester and subsequent saponification.

Friedelin is a ketone, and cerin an hydroxy ketone.<sup>9</sup> Cerin purified from chloroform can be sublimed in high vacuum without change in composition. The carbon and hydrogen content of this sublimed material corresponds to that demanded by the formula  $C_{30}H_{50}O_2$  first proposed by Thoms. Friedelin forms enol acylates without difficulty, and friedelin regenerated from the acetate, benzoate, or phenylacetate, has the carbon and hydrogen content demanded by the formula  $C_{30}H_{50}O$ .

Friedelin contains no active hydrogen, for it liberates no methane when treated with methylmagnesium halide; it does not form an ester with acetic anhydride alone or with acetyl chloride; benzoyl chloride at its boiling point yields a benzoate, and acetic anhydride with a trace of sulfuric acid yields an acetate. It is also possible to prepare the usual carbonyl derivatives of friedelin.<sup>9</sup> Since friedelin is a ketone, esterification must take place through the enol form, and this fact seems in accord with the drastic treatment to which friedelin must be subjected in order that esterification may occur. A sample of friedelin purified from the crude extracted material by a tedious series of recrystallizations, and another which had been purified through the phenylacetate, were identical in every respect with the product obtained by taking the friedelin prepared through the phenylacetate, converting it to benzoate and later regenerating friedelin by saponification.

The molecular weight of friedelin was determined by the saponification of certain esters and from the iodine content of the *p*-iodobenzoate. By studying a series of esters each differing from the preceding by a methylene group, we could be reasonably sure whether or not our experimental results were of sufficient accuracy to warrant assigning a definite formula to friedelin. The saponification equivalents of friedelin acetate, benzoate, phenylacetate, and  $\beta$ -phenylpropionate were determined. The mean value for the molecular weight of friedelin calculated from the data is 425, the calculated value for  $C_{30}H_{50}O$  is 426.

(9) Cf. papers II and III of this series.

By the method of Clemmensen, friedelin and cerin can both be reduced to a hydrocarbon. Melting points, analyses, and examination of the properties of the crystals indicate that a single chemical individual results from both substances. It is apparent, therefore, that cerin also possesses a nucleus of thirty carbon atoms. The molecular weight of cerin is, therefore, 442. Inasmuch as no ordinary method of combustion analysis yields results which are of sufficient precision to make possible the assignment of the number of hydrogen atoms in a molecule of this size, we are uncertain of the formulas assigned, *viz.*,  $C_{30}H_{50}O$  for friedelin and  $C_{30}H_{50}O_2$  for cerin, within the limits of 2H.

Neither friedelin nor cerin displays the colors given by sterols in the usual sterol tests, though cerin does give a very faint red color in the Liebermann-Burchard reaction. The fact that early investigators obtained positive tests for sterols when working with cerin was undoubtedly due to the presence of some impurity of sterol nature. In working up a considerable quantity of crude material, a gelatinous substance was obtained which gave strong colors in the Liebermann test. On the basis of analyses for carbon and hydrogen, it possesses the formula  $C_{28}H_{50}O_2$ . It adds bromine in carbon tetrachloride and gives strong sterol tests. Only 25 mg. of substance was isolated from 220 g. of crude product, and it is probable that this substance caused the sterol color reactions during tests of cerin by earlier investigators.

### Experimental

**Isolation of Cerin.**—Twenty-three kilograms of 20–30 mesh ground cork extracted with ethyl acetate in a batch extractor holding 2.3 kg. of cork, yielded about 1.5% of its weight of dry, nearly white, crude cerin and friedelin. Two recrystallizations of this material from chloroform sufficed to separate most of the friedelin. The less soluble cerin, after one recrystallization from benzene followed by two further recrystallizations from chloroform, was obtained in the form of fine silky laths which melted 247–251° with decomposition. A sample of this material was sublimed in high vacuum for analysis.

*Anal.* Calcd. for  $C_{30}H_{48}O_2$ : C, 81.74; H, 10.99. Calcd. for  $C_{30}H_{50}O_2$ : C, 81.37; H, 11.39. Calcd. for  $C_{30}H_{52}O_2$ : C, 81.01; H, 11.79. Found: C, 81.53, 81.58, 81.38; H, 11.68, 11.51, 11.56.<sup>10</sup>

Dr. S. B. Hendricks of the Fixed Nitrogen Laboratory of the U. S. D. A. has determined the properties of the crystals of friedelin and cerin. For cerin he found: habit, narrow laths, nearly needle-like; extinction, parallel, elongation negative;  $BX_a$ , character positive, angle less than friedelin and  $\perp$  to the same face; indices of re-

(10) All analyses by the micro methods of Pregl.

TABLE I  
 SOME FRIEDELIN ENOL ESTERS

Enol ester.....	Benzoate, C <sub>30</sub> H <sub>48</sub> OCOC <sub>6</sub> H <sub>5</sub>	Phenyl- acetate	$\beta$ -Phenyl- propionate	$p$ -Iodobenzoate C <sub>30</sub> H <sub>48</sub> OCOC <sub>6</sub> H <sub>4</sub> I	
Melting range, °C.	255-262	244-251	229-233	271-274 <sup>a</sup>	
Carbon, %	Calcd.	83.71	83.76	83.81	67.65
	Found	83.64	83.81	83.81	67.92
		83.62	83.89	83.87	67.87
Hydrogen, %	Calcd.	10.26	10.36	10.46	8.14
		10.33	10.53	10.65	8.29
	Found	10.33	10.46	10.50	8.01
		10.27			
Iodine, %	Calcd.			19.34	
	Found			19.33	
				19.18	

<sup>a</sup> Product prepared at 150-175°; not sublimed.

 TABLE II  
 SAPONIFICATION EQUIVALENTS OF FRIEDELIN ESTERS AND THE MOLECULAR WEIGHT OF FRIEDELIN

Ester	Saponification equivalents					Mol. wt. of friedelin					Calcd. for C <sub>30</sub> H <sub>48</sub> O	
	Found					Found						
Benzoate	523	533	530	526		530	419	429	426	422		426
Phenylacetate	537	547	548	547	545	542	419	429	430	427	426	426
$\beta$ -Phenylpropionate	559	556				554	427	424				426
Acetate	464	472	464			468	422	430	422			426

fraction,  $\alpha = 1.550$ ,  $\beta = 1.580$ ,  $\gamma = 1.615^{11}$  (BX<sub>a</sub> figure centered).

**Preparation of Enol Esters of Friedelin.**—By concentrating the chloroform filtrates from the first two recrystallizations of the crude material until solid separated from the hot solution, and then adding an equal volume of acetone, crude friedelin was precipitated as a crystalline powder; 10.0 g. of this "friedelin rich" material was heated with 20.0 ml. of benzoyl chloride for forty-five minutes in an oil-bath whose temperature was 150-185°. To the somewhat cooled reaction mixture was added cautiously 150 ml. of 95% alcohol. The lumps of solid which formed were broken up, the mixture was digested on the steam-bath for ten to fifteen minutes, and the warm suspension filtered. The solid was digested again with 100 ml. of alcohol, filtered and recrystallized from ethyl acetate-benzene. Seven g. of brilliant flat leaves were obtained, a sample of which was sublimed in high vacuum for analysis.

The procedure employed for the preparation of friedelin phenylacetate and  $\beta$ -phenylpropionate was similar. The melting ranges and analyses of these three esters are given in Table I.

**Saponification Equivalents of the Friedelin Esters.**—The method employed was that of Chargaff<sup>12</sup> with slight modifications. Forty to eighty mg. of ester was saponified by refluxing for thirty min. with 10 ml. of 0.05 *N* sodium *n*-propylate in *n*-propyl alcohol. The condenser was carefully washed with 10 ml. of water and the aqueous alcoholic suspension of friedelin was cooled and titrated. Table II gives a summary of the data, and also the values calculated therefrom for the molecular weight of friedelin.

**Preparation of Friedelin from the Friedelin Enol Esters.**—Ten grams of the phenylacetate was refluxed

for one hour in 500 ml. of *n*-propyl alcohol containing 0.6 g. of sodium. The system was homogeneous after ten minutes of boiling, whereupon crystalline material began to separate. The reaction mixture was cooled and filtered, yielding 6.4 g. of nearly white product. An additional 0.5 g. of friedelin was obtained by concentrating the filtrate. Recrystallization from ethyl acetate yielded perfectly white flat laths melting 255-261°.

Saponification of friedelin benzoate with alcoholic sodium hydroxide in pyridine yielded a product which after sublimation in high vacuum melted 255-261°.

*Anal.* Calcd. for C<sub>30</sub>H<sub>48</sub>O: C, 84.82; H, 11.40. Calcd. for C<sub>30</sub>H<sub>50</sub>O: C, 84.43; H, 11.82. Calcd. for C<sub>30</sub>H<sub>52</sub>O: C, 84.03; H, 12.23. Found: C, 84.33, 84.57, 84.59; H, 11.81, 11.87, 11.88.

The crystals possess the following properties: habit, broad laths; extinction, parallel, elongation negative. BX<sub>a</sub>, character positive, angle moderately large and  $\perp$  to the broad face of the lath; indices of refraction,  $\alpha = 1.550$ ,  $\beta = 1.575$ ,  $\gamma = 1.620^{11}$  (BX<sub>a</sub> figure centered); density 1.078.

Calculated from the root mean square index of refraction and the density, the value of *MR* is 132.0 using the Lorenz and Lorentz formula. The value of *MR* calculated,<sup>13</sup> assuming one double bond and a ketonic oxygen, is 131.9.

The presence of a double bond in the friedelin molecule is further indicated by the yellow color produced when the substance is treated with tetranitromethane.

**Isolation of a Sterol.**—Upon recovery of the alcohol from an alcoholic potassium hydroxide treatment of 220 g. of crude extract, there remained a gelatinous residue. This material was very soluble in alcohol and could be

(11) White light.

(12) *Z. physiol. Chem.*, **199**, 221 (1931).

(13) Swietoslawski, *THIS JOURNAL*, **42**, 1945 (1920).

precipitated from cold alcohol as a gelatinous solid. The bulk of the accompanying brown coloring matter was removed by repeated attempts to recrystallize the material from absolute alcohol. The white gel thus obtained was dried and sublimed in high vacuum. About 25 mg. of a product melting at 65–70° to a turbid liquid which cleared at 145° was obtained. The same behavior was observed on remelting the substance.

*Anal.* Calcd. for  $C_{27}H_{48}O_2$ : C, 80.12; H, 11.97. Calcd. for  $C_{28}H_{50}O_2$ : C, 80.31; H, 12.05. Found: C, 80.48, 80.46; H, 12.05, 12.12.

The substance adds bromine in carbon tetrachloride and gives strong color reactions when subjected to the sterol color tests. *Liebermann-Burchard Reaction*.—Rose-red color changing to purple, then to deep blue and finally to emerald-green.<sup>14</sup> *Salkowski Reaction*.—Yellow changing to a deep orange-red.<sup>15</sup> *Lifschütz Reaction*.—Faint pink changing slowly to a light purplish-brown.<sup>16</sup>

#### SPECIFIC ROTATIONS OF CERIN, FRIEDELIN AND SEVERAL FRIEDELIN ENOL ESTERS

Compound	C	Temp., °C.	$[\alpha]_{461}$
Cerin	0.954	25	-44.5
	1.18	27	-42.4
Friedelin	3.046	23	-29.4
	7.257	20	-29.4
Friedelin benzoate	1.804	25	+66.2
Friedelin phenylacetate	1.654	26	+57.1
Friedelin $\beta$ -phenylpropionate	1.022	23	+52.8
Friedelin <i>p</i> -iodobenzoate	1.258	25	+61.2

**Preparation of the Parent Hydrocarbon from Friedelin.**—Pure friedelin was reduced in acetic acid solution by means of zinc amalgam and hydrochloric acid with the addition of a small quantity of xylene. The product obtained melts 245–249° and is a hydrocarbon.

*Anal.* Calcd. for  $C_{30}H_{52}$ : C, 87.29; H, 12.71. Found: C, 87.08, 87.12; H, 12.62, 12.64.

**Preparation of the Parent Hydrocarbon from Cerin.**—A process similar to the above yielded a product identical with the hydrocarbon obtained from friedelin.

*Anal.* Calcd. for  $C_{30}H_{52}$ : C, 87.29; H, 12.71. Found: C, 87.32; H, 12.57.

The hydrocarbon does not add bromine, and gives a faint coloration with tetranitromethane.

The crystals of both hydrocarbons have the same properties: both are optically positive with moderate birefringence and moderate optic axial angle; both have a value for  $\beta$  = intermediate refractive index = 1.573. The extinction angle on apparently monoclinic plates from the *c* axis for cerin is 18° 45' and for friedelin 17° 56' with a probable error of  $\pm 1^\circ$  from the mean.

This substance gives a yellow color when dissolved in tetranitromethane, indicating the presence of an unsaturation. Apparently reduction by the Clemmensen method does not saturate what we suspect is an intranuclear double bond. It is quite unlikely, therefore, that this double bond is conjugated with the carbonyl group in friedelin.

(14) Liebermann, *Ber.*, **18**, 1894 (1885).

(15) Salkowski, *Z. physiol. Chem.*, **57**, 523 (1908).

(16) Lifschütz, *Ber.*, **41**, 252 (1908).

## Conclusions

It is apparent from our work that none of the earlier investigators in the field, excepting Thoms, worked with pure cerin, and that the friedelin of Istrati and Ostrogovich was a mixture.

The empirical formula of friedelin is  $C_{30}H_{50}O$ , that of cerin  $C_{30}H_{50}O_2$ , both plus or minus two H. The precision of the saponification equivalent determinations leaves no doubt concerning the presence of thirty carbon atoms in the friedelin molecule. The molecular refraction of friedelin is likewise in accord with the formula  $C_{30}H_{50}O$ . The hydrocarbon to which friedelin is related is  $C_{30}H_{52}$  and our analytical results are in accord with this formula. Because of the paucity of hydrogen atoms, the apparently saturated character, and high melting point of friedelin, the substance must be polycyclic in nature. Basing our conclusion on the agreement between the observed value for the molecular refraction of friedelin and that calculated for one double bond and a ketonic oxygen, we arrive at a formula in which there must be 4 cycles and a double bond. Admittedly the assumption of the presence of a double bond is based solely on the molecular refraction data and the color reaction with tetranitromethane. Addition of bromine, if it does occur, takes place with more difficulty than substitution, and hydrogenation does not occur through use of a platinum catalyst at room temperature and moderate pressure. Such behavior of an intranuclear double bond is not unknown; one of the double bonds of ergosterol behaves in much this fashion.

The conversion of cerin and friedelin into the same hydrocarbon,  $C_{30}H_{52}$ , is not only in accord with this picture, but is excellent evidence that both cerin and friedelin have the same carbon skeleton. The fact that the unsaturation of friedelin still persists in the hydrocarbon produced by Clemmensen reduction makes it extremely unlikely that the intranuclear double bond of friedelin is conjugated with the carbonyl group. If such were the case, this double bond would certainly be saturated during the lengthy reduction of friedelin to the hydrocarbon.

## Summary

1. The preparation of pure friedelin and cerin has been described.
2. The molecular weight of friedelin has been determined by the saponification of a number of different enol esters.

3. Friedelin benzoate, phenylacetate,  $\beta$ -phenylpropionate, and *p*-iodobenzoate have been prepared.

4. Friedelin and cerin have been reduced by the Clemmensen method to the same hydro-

carbon,  $C_{30}H_{52}$ .

5. Friedelin is probably a polynuclear substance containing four rings and one double bond.

COLLEGE PARK, MD.

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## Action of Inorganic Bases on Isobutyl Bromide<sup>1</sup>

BY H. E. FRENCH AND W. H. WADE

In a previous communication<sup>2</sup> the action of dilute aqueous solutions of various inorganic bases on secondary and tertiary butyl bromides was discussed. This work has now been extended to cover the behavior of isobutyl bromide with the same reagents. This latter substance is of interest because of the tertiary hydrogen attached to the carbon atom adjacent to the one holding the bromine. Such hydrogens are far more reactive toward certain reagents than are primary and secondary hydrogens. Toward aqueous inorganic hydroxides, any such reactivity on the part of isobutyl bromide would result in the formation of butene. The extent of such a reaction under various conditions is of interest.

Isobutyl bromide shows a marked tendency to rearrange to the tertiary bromide. This rearrangement may take place in either the gaseous or liquid phase, often at temperatures near 100°, and is catalyzed by a number of different substances.<sup>3</sup> It therefore seemed a possibility that rearrangement might take place with the isobutyl bromide, with the rearranged product entering into the reaction with the reagent. The investigation of such questions should yield information as to the most favorable conditions for the hydrolysis of alkyl halides.

### Reagents, Apparatus and Procedure

The isobutyl alcohol was the best grade obtainable from the Eastman Kodak Company, and before use was distilled through a 90-cm. fractionating column filled with glass rings. The boiling point was 107.3°. The alcohol was converted into the bromide by the method described by Reynolds and Adkins.<sup>4</sup> Silver hydroxide was prepared as previously described, and the other reagents were of the

quality used in the previous work. The apparatus and procedure were essentially those used in the earlier investigation,<sup>2</sup> though in the present work the alcohol obtained by hydrolysis was isolated and identified in each case. This was done in the following manner. The butyl alcohol solutions obtained by distillation of the barely acidified reaction mixtures were redistilled through a 90-cm. column filled with glass rings. Isobutyl alcohol forms a constant boiling mixture with water at 89.82° at 760 mm.<sup>5</sup> In each distillation 8–10 cc. was distilled over, using a graduated tube of about 1 cm. diameter for receiver. In all cases two layers formed, but anhydrous potassium carbonate was added to decrease the solubility of the isobutyl alcohol. From 0.20 to 1.95 cc. of alcohol was obtained from the various reactions. Yields calculated from these volumes generally ran 15–20% below the theoretical yields calculated from the olefin and metallic halide determinations. In each case the alcohol was converted into the 3,5-dinitrobenzoate, and the melting point was determined.

As in the previous work, the olefin was determined by means of an Orsat gas analysis apparatus, while the metallic bromide was determined volumetrically, except for the silver bromide, which was weighed directly.

From two to three runs were made at each concentration and temperature for each of the reagents. As in the previous work, the variation in the yield of olefin between duplicate reactions never exceeded 2.2%, and was usually considerably less than that amount.

The time of reaction was usually ten hours, but in no case did reaction go to completion. There was, however, no reaction between unchanged isobutyl bromide and the water on distillation of the neutralized reaction mixtures. This was demonstrated by distilling a mixture of 12 g. of isobutyl bromide and 500 cc. of water and testing the residue for halogen. The test was negative.

### Summary of Reactions

In each case 0.087 mole of the isobutyl bromide was added to the reaction mixture. The yields are based on the amounts of the alkyl bromide which reacted, as determined from the analyses for the metallic bromide.

### Discussion

As was expected, the isobutyl bromide reacted much more slowly than did the secondary and

(1) This paper is an abstract of the thesis submitted by Mr. Wade in partial fulfillment of the requirements for the degree of Master of Arts at the University of Missouri.

(2) French, McShan and Johler, *THIS JOURNAL*, **56**, 1346 (1934).

(3) Michael, Scharf and Voigt, *ibid.*, **38**, 653 (1916); Whitmore and Lux, *ibid.*, **54**, 3448 (1932).

(4) Reynolds and Adkins, *ibid.*, **51**, 285 (1929).

(5) Young, *J. Chem. Soc.*, **81**, 727 (1902).