# Clathrates of Sodium Warfarin

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From a combination of X-ray diffraction and chemical and analytical studies, it has now been established that sodium warfarin, crystallized from propanol-2, is the host molecule for a series of clathrates in which propanol-2 and water are the guest molecules. It has been established that a continuous series of compositions can be made in which the ratio of sodium warfarin to propanol-2 and water may vary from 8:4:0 to 8:2:2 and still possess a monoclinic space lattice whose space group is $P2_1/c$ and whose lattice dimensions are $a = 15.4$ Å., $b = 11.4$ Å., and $c = 22.7$ Å. with $\beta = 107°$.

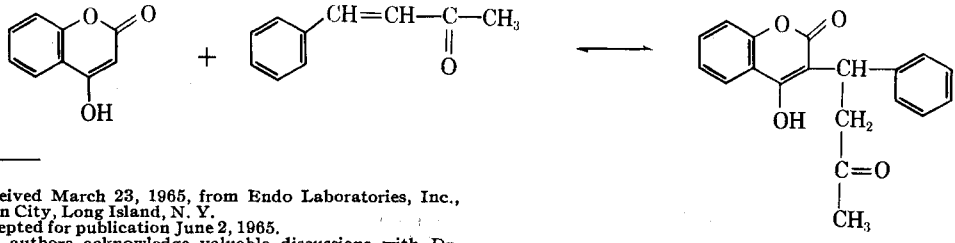
THE PURPOSE of this investigation was to elucidate the nature of the complexes which formed when sodium warfarin[1] was crystallized from anhydrous propanol-2 and from propanol-2 solutions containing some water. This crystalline material had been the subject of a patent issued to Link and Schroeder (1). In that patent they disclose a process for making such crystals in sizable amounts with a composition approximating 3 moles of sodium warfarin to 1 mole of propanol-2.

The authors were directed into this investigation by the need to prepare a suitable control procedure, not only for the manufacture of the pure substance, but also for the various formulations which were being prepared.

Sodium warfarin, *per se*, is an item already described in the "United States Pharmacopeia" XVI. There it is described as a crystalline substance, which it is not. Indeed, the primary object of the Schroeder-Link investigations was to devise a method for preparing a crystalline product as a means for eliminating a degradation product present in the amorphous material and determined by the U.S.P. color in alkaline solution test (2).

## DISCUSSION

**Method of Manufacture.**—Warfarin was first produced by a Michael addition (3) of 4-hydroxycoumarin to the $\alpha,\beta$-unsaturated ketone, benzalacetone—*viz.*,




The commercial method employed (4) consists of refluxing for 3 hr. 1 mole of each of the two reagents and 0.1 mole of triethylamine, together with 3.5 times their combined weight of water. A heavy precipitate results which is partially purified by washing with hot benzene. It is finally purified by dissolving in strong alkali and extracting with carbon tetrachloride. The purified warfarin is precipitated from solution with acid, washed with water, and dried. Yields of 75 to 90% are usual.

The formation of the sodium salt is effected by the addition of concentrated sodium hydroxide solution to an aqueous slurry of powdered warfarin with continuous agitation (5). Insufficient alkali is added so that an excess is avoided. This aqueous solution containing some unreacted warfarin and residual insoluble impurities is now treated with activated charcoal and then filtered. The clear aqueous solution resulting is concentrated to a thick syrup under reduced pressure. The syrupy material is transferred to trays and further dried in vacuum ovens with occasional grinding of the solidified mass to produce a fine powder and to assist further in the dehydration step. The final product, as prepared, conforms to the requirements of U.S.P. XVI for sodium warfarin in all respects but one. It is not crystalline, but rather, it is an amorphous powder.

Many attempts have been made to crystallize this material from solvents in which it is soluble. These include, among others, the normal lower alcohols, ethyl acetate, acetone, and *N,N*-dimethylformamide, to name a few. In all cases, after removal of the solvents, the original amorphous material was reclaimed along with its usual impurities. However, in their investigations of this problem, Schroeder and Link made the unantici-

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[1] Synthetic hypoprothrombinemia inducing agent 3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium salt. Marketed as Coumadin Sodium by Endo Laboratories, Garden City, Long Island, N. Y.

pated discovery that when sodium warfarin was in solution with propanol-2, a crystalline complex was formed which could be separated devoid of its impurities as indicated by very substantial reduction in the

absorbance obtained when the U.S.P. color in alkaline solution test is applied to it.

The method of crystallization which they employed was to concentrate the filtered aqueous sodium warfarin solution mentioned above to a syrupy stage, where the concentration of the salt would be about 21 to 22% by weight. Then they would add various amounts of propanol-2 and distil away the water and alcohol as the constant boiling mixture. Successive increments of the propanol-2 were added, followed by distillation until so much of the alcohol had been added that crystals of a sodium warfarin·propanol-2 complex could be seen forming in the flask, after which the remaining alcohol was distilled off under reduced pressure. The crystalline material which they formed contained about one-third of a mole of alcohol for each mole of sodium warfarin and had a propanol-2 content of about 5.7%.

Even though the propanol-2 boils at 82.4° at 760 mm. pressure, they found that it took about 24 hr. of heating at 145° and 1 mm. of pressure for its removal. A rapid release could be effected by heating the complex to 230°, whereupon it melts, decomposes, and releases the alcohol. If the propanol-2 is removed by heating at 145°, the crystalline structure is destroyed, and the material reverts to amorphous sodium warfarin and some decomposition products.

The first intimation that the crystalline complex realized in the Schroeder and Link process (1) might vary in propanol-2 composition came from the analytical control data of successively manufactured batches. On the material dried to constant weight, it was anticipated that the sodium warfarin content would run 94.3 ± 0.5%. Although most lots assayed within range, assays occasionally were obtained below the lower limit and could not be accounted for. However, when the late Professor Fankuchen and his assistants made a single-crystal X-ray diffraction study, a contradiction was uncovered between the data thus obtained and the chemical data which we had. Analysis of the X-ray data showed that the unit cell had some multiple of four sodium warfarin molecules in it and this was incompatible with a 3:1 complex.

Accordingly, it was decided to develop a method for the direct assay of the alcohol content of these crystalline complexes and apply it to our production lots of the material as well as to the special crystals which were prepared for the X-ray diffraction study.

**Determination of Propanol-2.**—The procedure for the determination of propanol-2 which was first tried was that described by Frisone (6) for the determination of this alcohol in dextran.

In that procedure the crystalline powder is dissolved in water, the propanol-2 distilled out of the solution and collected in an acid dichromate solution. There it is gently refluxed to oxidize the alcohol to acetone. After making the solution alkaline, the acetone is distilled into an alkaline hypoiodous acid solution where it is iodinated to iodoform according to the over-all reaction:

$$CH_3COCH_3 + 3I_2 + 4NaOH \rightarrow CHI_3 + CH_3CO_2Na + 3NaI + 3H_2O$$

Using the above procedure, unsatisfactory results were obtained at first.

In examining the literature a little further, the authors found an excellent study by Morgan *et al.* (7), who had systematically investigated the order of addition of the reagents, the effect of their relative strengths, and rates of addition. To obtain quantitative results, it is important (*a*) that the acetone be premixed with the alkali before the iodine is added and (*b*) that large excesses of alkali must be used (at least twentyfold excess over that required by the stoichiometry of the reaction) in order to assist in the enolization of the acetone and to catalyze the carbon–carbon bond fission.

The procedure which was finally adopted is described in the following paragraphs.

Dissolve 800 mg. of dry crystalline sodium warfarin·propanol-2 complex in about 25 ml. of water in a 50-ml. volumetric flask, add 3 ml. of 4 *N* sulfuric acid, and bring to volume with water. Filter the solution through Whatman No. 5 filter paper. Pipet a 10.0-ml. aliquot into a 250-ml. round-bottom flask and dilute to 40 ml. with water. Connect the flask to a condenser and through the top add 30 ml. of an oxidizing solution consisting of 10 Gm. of $K_2Cr_2O_7$, dissolved in 100 ml. of 20% $H_2SO_4$.

Heat on a steam bath for 15 min. and then rinse the inside of the condenser with 10 ml. of ice cold water and immerse the flask, with the condenser attached, into an ice cold bath. When the solution is cold, disconnect the flask and connect it to a West condenser and distil 60 ml. collecting the distillate in a 250-ml. iodine flask containing 20 ml. of 2 *N* NaOH. The iodine flask should be immersed in an ice bath. Stopper the flask and warm to room temperature. While swirling the contents of the flask, add 20.0 ml. of 0.1 *N* iodine dropwise from a buret. Stopper the flask and hold for 30 min. Finally, add enough 6 *N* HCl to liberate the excess iodine and titrate with sodium thiosulfate. Each milliliter of 0.1 *N* iodine consumed is equal to 1.001 mg. of propanol-2.

Some of the results obtained with the above method are presented in Table I.

The 10-mg. standard which was used was made by weighing out a 1.00-Gm. sample of absolute propanol-2 and transferring it to a 1-L. volumetric flask followed by diluting to mark with water.

TABLE I.—ASSAY FOR PROPANOL-2 IN VARIOUS STANDARDS AND PRODUCTION SAMPLES

| Sample Type | % Recovery |
|---|---|
| 10 mg. standard | 99.5 |
| | 98.5 |
| | 100.6 |
| | 99.5 |
| | 100.0 |
| | 100.1 |
| **Production lots of crystalline complex** | **% Propanol-2** |
| 63126 | 5.99 |
| 62154 | 6.24 |
| 62155 | 6.64 |
| 63540 | 5.74, 5.65 |
| Special crystals made from propanol-2 dried over sodium | 8.2, 8.15, 8.16, 7.62, 7.62 |
| Crystallized without special precautions | 6.4 |
| | 5.28 |
| From 10% water | 4.99 |
| From 15% water | 4.59 |
| From 17% water | 4.45 |

TABLE II.—CALCULATED COMPOSITIONS FOR VARIOUSLY ASSUMED STRUCTURES

| Compn. $H_8G_xG'_y$ | Space Lattice, Mol. Wt. | Mol. Wt. | % Propanol-2 | % $H_2O$ | % Na Warfarin |
|---|---|---|---|---|---|
| 8:4:0 | 2880 | 360 | 8.33 | 0.00 | 91.67 |
| 8:3:1 | 2838 | 354.75 | 6.25 | 0.33 | 93.42 |
| 8:2.67:1.33 (Schroeder-Link compn.) | 2824 | 353 | 5.67 | 0.85 | 93.48 |
| 8:2:2 | 2796 | 349.5 | 4.30 | 1.28 | 94.42 |
| Compn. | % C | % H | % O | % Na | |
| 8:4:0 | 68.3 | 5.27 | 20.0 | 6.37 | |
| 8:3:1 | 68.1 | 5.15 | 20.3 | 6.48 | |
| 8:2.67:1.33 | 68.0 | 5.10 | 20.35 | 6.51 | |
| 8:2:2 | 67.9 | 5.02 | 20.6 | 6.55 | |

Ten-milliliter aliquots of this solution were taken. The results are typical.

Also shown are the results of assaying different production lots of the crystalline complex using the crystallizing conditions of the Schroeder-Link patent (1).

Finally, there are presented the assay results on a group of crystalline complexes prepared under a variety of conditions. It is seen that the crystal may have as much as 8.2% propanol-2 in it, although that content can vary downward to 4.45. The complex with the 8.2% propanol-2 content was submitted for a single-crystal X-ray diffraction study. At the time it was submitted, we had not yet developed the propanol-2 assay procedure and consequently expected that it would have a composition of about 5.7%, corresponding to the 3:1 ratio.

The crystalline sodium warfarin–propanol-2 complex prepared for the X-ray diffraction study was made somewhat differently from the procedure described above. In this instance every attempt was made to exclude water from the system. The alcohol was dried over sodium metal and then distilled in dried glassware. Anhydrous sodium warfarin, *i.e.*, dried to constant weight at 105°, was dissolved in boiling propanol-2. When it appeared that the solution was saturated, the refluxing of the propanol-2 was stopped and the clear layer of solution was decanted through a funnel containing a pledget of cotton into a separate flask where it was allowed to cool overnight, after which the excess solution was decanted and further concentrated. From this concentrated solution a second group of relatively large crystals was obtained in the same way as the first. After a decantation of the excess solution, the crystals were washed several times with cold anhydrous propanol-2 to free them of any adhering mother liquor. They were dried to constant weight, which usually meant they were heated for 3 hr. at 105°. Examined under the microscope, they appeared as monoclinic needles, approximately one-third of a millimeter in length and between a tenth and a twentieth of a millimeter in width. The other crystals which were prepared in that series were made without special precautions for dryness, or were made from water–propanol-2 solutions listed. As the percentage of water increased in the crystallizing medium, the size of the crystals diminished, and their appearance as crystals became less and less satisfactory. With 15 and 17% water the material obtained showed no evidence of crystallinity by microscopic examination.

**X-Ray Diffraction Studies.**—A number of diffraction patterns were taken along the needle axis of the crystal (called *b*) with a Weissenberg camera. From the zero-layer photograph and from an oscillation photograph about the needle axis, the reciprocal lattice was determined. From this it was established that the dimensions of this monoclinic crystal were: $a = 15.4 \pm 0.2$ Å.; $b = 11.4 \pm 0.2$ Å.; $c = 22.7 \pm 0.2$ Å.; $\beta = 107°$.

It was further established that the space group was primitive with a *c* glide perpendicular to the *b* axis. It was also established that there was a $2_1$ screw axis parallel to the *b* axis. Thus, the space group was uniquely determined as $P2_1/c$. Knowing the lattice dimensions, the volume of the unit cell could be calculated—*viz.*, $a \times b \times c \sin \beta = 3828 \pm 134$ cu. Å.

By reference to X-ray crystallography tables (8) it is seen that for the $P2_1/c$ structure, the number of sodium warfarin molecules per unit cell must be some multiple of 4. However, our choice of the multiple is restricted by the fact that the density of the crystal would increase linearly with an increase in this multiple. A determination of the density of these crystals was made at 25°, using carbon tetrachloride–benzene mixtures whose specific gravities were adjusted in the range from 1.20 to 1.40. It was found that the density was between 1.28 and 1.30. If the assumption is made that in these crystals there is one propanol-2 molecule for each two sodium warfarin molecules, as indeed proved to be the case, the molecular weight of the presumed complex would be 360.

The density of a crystal is given by the relation

$$d = \frac{\text{mol. wt.} \cdot n}{VN}$$

$$n = \frac{d \cdot V \cdot N}{\text{mol. wt.}}$$

where

$d$ = density = 1.29
mol. wt. = molecular weight = 360
$n$ = number of sodium warfarin molecules in the space lattice
$V$ = volume of space lattice in cubic Angstroms = $3828 \pm 134 \times 10^{-24}$
$N$ = Avogadro's number = $6.02 \times 10^{23}$

Substituting these values into the above relation we see that $n = 8$.

At this stage of the investigation it was assumed that we were dealing with a clathrate in which the host molecule was sodium warfarin with propanol-2 and water as guest molecules. Thus, in the space lattice we would have a composition $H_8G_xG'_y$, where $H$ is the sodium warfarin, $G$ and $G'$ are propanol-2 and water, respectively, and $x$ and $y$ are small

whole numbers such that $x + y = 4$, and with $y$ any value between zero and 2. In Table II are presented some calculated percentage compositions which would be obtained for various assumptions.

The interesting things to note are as follows. (*a*) As the composition changes from 8:4:0 to 8:2:2 the propanol-2 content varies from 8.33 to 4.30, and this compares favorably with the range of 8.2 to 4.45 which was found experimentally in Table I. (*b*) The elemental composition percentages do not change appreciably thus precluding the use of such assays for arriving at any conclusions concerning these crystals. (*c*) The water content of the lattice should vary between 0.0 and 1.28%.

It became of interest, therefore, to determine whether the water content did indeed vary as indicated and whether the crystalline character and structure remained unchanged over this range of compositions.

The water determinations were made by a Karl Fischer titration, using a technique and apparatus previously described (9). The sample most closely corresponding to the 8:4:0 composition gave values of 0.10, 0.12%, while the composition which was closest to the 8:2:2 composition assayed 1.14% in good agreement with expectation. Intermediate compositions showed intermediate percentages.

Powder diagrams were made of a number of compositions, using a Norelco diffractometer. Copper $K\alpha$ radiation was used along with a nickel filter to give radiation of 1.540 Å. The sample was irradiated continuously while the sample and counter were rotated in synchronism to read diffraction effects over ranges of $2\theta$ from 9° to 31°. Some of the results of those scans are presented in Fig. 1.

Proceeding from the top of the figure downward, the scans are of crystals whose compositions approximated the ratios 8:4:0, 8:3:1, and 8:2:2, with propanol-2 contents of 8.2, 6.4, and 4.45%, respectively.

What is observed is that the two upper diffraction patterns are identical in the angular position of all

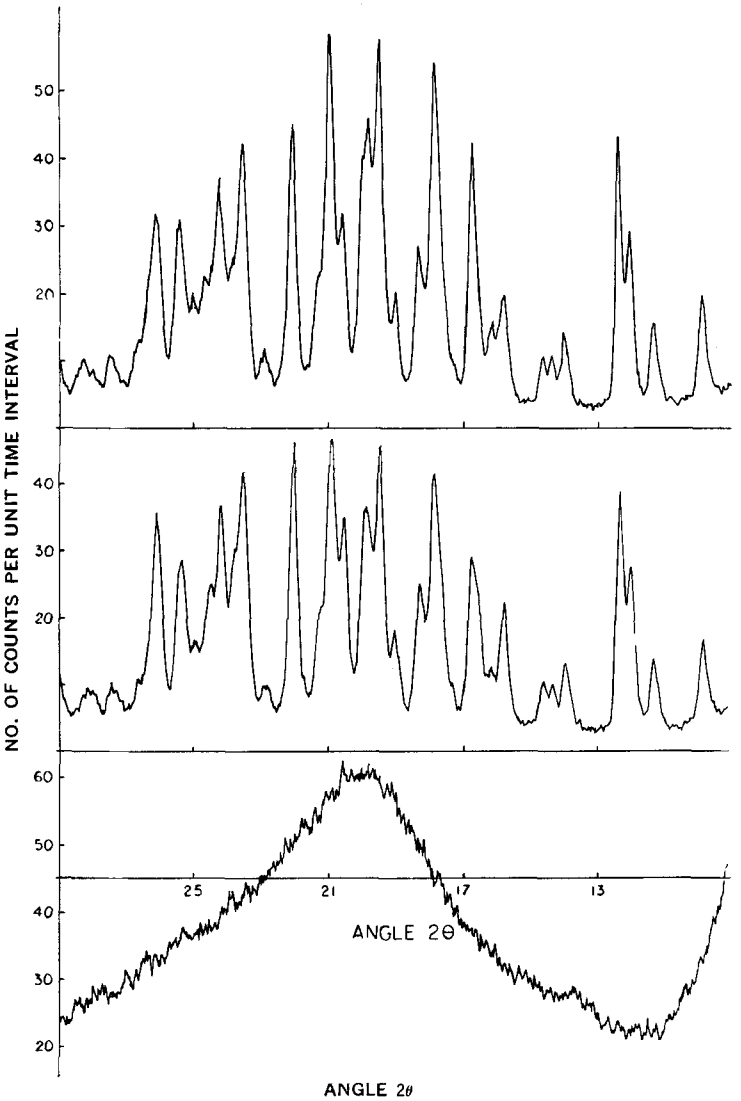


Fig. 1.—Key: *A*, 8:4:0 structure; *B*, 8:3:1 structure; *C*, 8:2:2.

the peaks. The peak heights are of no significance in this particular analysis. The important feature is that the spacing of the crystal planes is quantitatively the same for both compositions, indicating identical crystal structures, even though a molecule of water has been substituted for one of propanol-2 molecules. By substituting water for two of the propanol-2 (see bottom curve), the lattice is so weakened that definite Bragg spacings are not observed. The crystallites, if present, are now so small that the peak heights broaden out to reveal a single envelop in the $13° < 2\theta < 27°$ region with a broad maximum at 20°, much as would be expected.

The addition of more water to the clathrate leads to its collapse, as revealed by the authors' inability to get any crystals at all when the propanol-2 content is reduced below 83% in the crystallizing medium.

## CONCLUSION

It now seems clear that when Schroeder and Link crystallized out a complex that appeared to have the 3:1 composition they had fortuitously chosen a set of conditions relatively easy to reproduce, which repeatedly gave them that composition. In the authors' production laboratory there is no difficulty in keeping the composition between such limits as 5.25 to 6.25% propanol-2.

The 5.72% composition which Schroeder and Link found may be inferred to be crystals with space lattices of the three different compositions, *i.e.*, 8:4:0, 8:3:1, and 8:2:2, taken in such proportions that the 8:2.67 composition results.

To see the detail of this let us assign the letters $A$, $B$, and $C$ to the compositions 8:4:0, 8:3:1, and 8:2:2, respectively. It follows from this that the number of propanol-2 molecules in any given crystal is equal to

$$4A + 3B + 2C$$

It also follows that the number of space lattices in any crystal is equal to $A + B + C$.

For the Schroeder-Link composition, the ratio of the number of propanol-2 molecules to the number of space lattices will equal 8/3—*viz.*,

$$\frac{4A + 3B + 2C}{A + B + C} = 8/3$$

which may be simplified to

$$4A + B - 2C = 0 \qquad \text{(Eq. 1)}$$

In solving this equation the values assigned to the letters must be discrete integers since we cannot have fractions of a molecule in any lattice.

The combinations of $A$, $B$, and $C$ which will fit Eq. 1 now can be calculated easily. The first solution of that equation which is obtained listing the values of $A$, $B$, and $C$ in that order is 0:2:1. This means that in every domain of three space lattices, two will have a composition of 8:3:1, and one will have a composition of 8:2:2. There is no requirement here for the 8:4:0 composition in any of the space lattices in that domain.

The next series of solutions involves having one 8:4:0 space lattice in the domain. The solutions that are obtained then are given in the following set:

| | |
|---|---|
| 1:0:2 | 1:6:5 |
| 1:2:3 | 1:8:6 |
| 1:4:4 | 1:10:7, etc. |

This set may be extended by merely increasing $B$ by increments of 2 and $C$ by increments of unity. Thus in domains containing one 8:4:0 lattice, a large but not infinite number of combinations give the Schroeder-Link composition. Although the number of solutions to Eq. 1 is infinite, the number of combinations is not, because it is limited by the crystal size.

Another set can be prepared for domains containing two of the 8:4:0 space lattices in it. The set with this assumption is as follows:

| | |
|---|---|
| 2:2:5 | 2:14:11 |
| 2:6:7 | 2:18:13 |
| 2:10:9 | 2:22:15, etc. |

The set may be further extended by increasing $B$ in increments of 4 and simultaneously $C$ by increments of 2. It will be noticed that the combinations 2:0:4, 2:4:6, 2:8:8, 2:12:10, 2:16:12, etc., do not appear in this set because these are combinations that appear in the first set as 1:0:2, 1:2:3, 1:4:4, 1:6:5, and 1:8:6. A third and fourth set may also be prepared by assuming three or four of the 8:4:0 space lattices in the crystal domain. They are

| | |
|---|---|
| 3:2:7 | 4:2:9 |
| 3:4:8 | 4:6:11 |
| 3:8:10 | 4:10:13 |
| 3:10:11 | 4:14:15 |
| 3:14:13 | 4:18:17 |
| 3:16:14, etc. and | 4:22:19, etc. |

Omitted as repetitious of combinations in lower sets are

| | |
|---|---|
| 3:0:6 | 4:0:8 |
| 3:6:9 | 4:4:10 |
| 3:12:12 | 4:8:12 |
| 3:18:15 | 4:12:14, etc. |

A large but not infinite number of additional higher sets can be assembled for the Schroeder-Link composition. In all of these sets except two domains—*viz.*, 0:2:1 and 1:0:2—all three space lattice compositions are required.

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