TABLE IV

Wave length, μ	Wave length, μ	Wave length, μ	Wave length, μ
2.55	4.12	6.10	9.17
2.75	4.24	6.20	9.63
3.08	4.34	6.32	10.08
3.13	4.63	6.79	10.32
3.34	4.98	6.94	10.60
3.39 s	5.21	7.15	10.95 s
3.54	5.42	7.37	11.23
3.59	5.62	7.59	11.43
3.65	5.71	8.17	12.51
3.86	5.78	8.30	14.85

s, indicates shoulder on side of stronger band.

measurements were taken at two concentrations, 0.0298 and 0.0993 g. per liter, designated (1) and (2), respectively, in the figure. The cells used measured 1.00 cm. in thickness.

Acknowledgments.—The crystallization apparatus described in the foregoing was designed and constructed by D. W. Scott of the Thermodynamics Section of this Station for another purification problem, and only minor modifications were necessary to adapt it for use with cyclooctatetraene.

Summary

The density, viscosity, refractive index and the infrared and ultraviolet absorption spectra of a purified sample of cycloöctatetraene are presented. The purification procedure and the apparatus used are described, and the equations relating to viscosity–temperature, density–temperature and refractive index–wave length over the ranges investigated are given.

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[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION, COMMERCIAL SOLVENTS CORPORATION]

Hexamminecobalt(III) Benzylpenicillinate[1]

BY ROBERT W. SHORTRIDGE AND CECIL CARR

Recent publications from the University of California College of Pharmacy[2] have indicated that the divalent cobalt ion potentiates the antibacterial activity of penicillin *in vitro* and *in vivo*. It therefore became of some interest to prepare a compound or compounds containing both cobalt and penicillin. Of particular interest were compounds containing various complex ions of trivalent cobalt, from the standpoints of stability and the above-mentioned potentiating effect.

When potassium benzylpenicillinate and hexamminecobalt(III) chloride in a 3:1 molar ratio were dissolved in no more than enough water to make a practically saturated solution of the latter salt, no precipitation occurred. Addition to this solution of a moderate amount of chloroform caused almost immediate formation of an orange-red oil, intermediate in density between the aqueous and the chloroform layer, difficultly soluble in the former, and insoluble in the latter.

Upon continued stirring and rubbing, the oil crystallized, usually within thirty minutes or so, to orange platelets with a pearly luster and showing parallel extinction. These crystals, while insoluble in chloroform, were preferentially wet by it, and in the presence of sufficient chloroform remained suspended therein.

Vapor pressure–composition measurements on the product indicated that it contained chloroform and hexamminecobalt(III) benzylpenicillinate in a 2:1 molar ratio. Its equilibrium vapor pressure in the presence of a little of the chloroform-less salt was 70 mm. at room temperature. It lost chloroform upon drying in air or *in vacuo*, and its elementary composition attained as a limit that of hexamminecobalt(III) benzylpenicillinate trihydrate. Both benzylpenicillinic acid diisopropyl etherate and hexamminecobalt(III) oxalate were prepared in good yield from this product.

A reaction similar to that described above occurs when methylene chloride is substituted for chloroform; an orange-red oil is formed which slowly crystallizes. Upon drying, hexamminecobalt(III) benzylpenicillinate trihydrate is formed.

Hexamminecobalt(III) benzylpenicillinate trihydrate is an orange-red crystalline powder whose melting point is not sharp, but whose decomposition point is around 150°. Its microbiological activity against *Staphylococcus aureus* by the standard cylinder–plate assay approaches closely to the value of 1467 units/mg., calculated from the defined value of 1667 units/mg. for sodium benzylpenicillinate. Any *in vitro* effect of the hexamminecobalt(III) ion on the activity would be reflected in a deviation from the calculated value; therefore, within the limits of accuracy of the test, there is no such effect. *In vivo* tests are being performed and will be reported elsewhere.

The microbiological activity of various samples prepared remained constant during several months' exposure to laboratory conditions, indicating stability of an order comparable to that of the crystalline alkali metal penicillinates.

(1) This work was presented before the Organic Division at the 116th Meeting of the American Chemical Society at Atlantic City, New Jersey, September, 1949.

(2) (a) Pratt and Dufrenoy, *J. Bact.*, **55**, 727 (1948); (b) Pratt, Dufrenoy and Strait, *ibid.*, **55**, 75 (1948); (c) Pratt and Dufrenoy, *ibid.*, **54**, 719 (1947).

The product is rather difficultly wettable by water, but is slowly soluble. It is much less soluble than the alkali metal penicillinates, but more soluble than procaine benzylpenicillinate.

Experimental

Preparation of Hexamminecobalt(III) Benzylpenicillinate Trihydrate.—Hexamminecobalt(III) chloride[3] (1.34 g.) and 5.59 g. of potassium benzylpenicillinate of over 95% purity were dissolved in 25 ml. of water. With continual stirring, 100 ml. of chloroform (U. S. P.) was added. An orange-red oil separated almost immediately, and, upon continued stirring and scratching with a glass rod, crystallized to orange platelets. These, being suspended in the chloroform layer, were readily washed free of impurities in a separatory funnel with three 25-ml. portions of water, and were then filtered and allowed to dry in air. In repeated preparations of this compound by the above method or slight variations of it, the yield averaged around 4 g. (65%) of the compound assaying satisfactorily close to the calculated 1467 units/mg.

Substitution of methylene chloride for chloroform in the above reaction gave identical results.

The compound was relatively soluble in wet butanol, and could be recrystallized therefrom by reduced pressure distillation of the butanol–water azeotrope; the butanol thus dried deposited crystals of pure hexamminecobalt(III) benzylpenicillinate trihydrate.

Anal. Calcd. for $C_{48}H_{75}O_{15}N_{12}S_3Co$: C, 47.4; H, 6.22; N, 13.8; S, 7.92; Co, 4.85. Found: C, 47.5; H, 6.19; N, 13.8; S, 8.10; Co, 5.00.

Determination of Composition of the Hexamminecobalt(III) Benzylpenicillinate–Chloroform Complex.—A sample of the complex including excess chloroform was weighed into a glass cup attached to a three-way stopcock. The weight of the stopcock, cup and sample was obtained.

One arm of the three-way stopcock was connected to a closed end manometer and the other to a vacuum pump. The sample was frozen in an acetone–Dry Ice slurry, and the system evacuated. The stopcock was turned to connect only the sample and manometer. The vapor pressure was read when room temperature (approximately 28°) was obtained.

The manometer was then alternately connected to the vacuum and the sample. When a small amount of the chloroform was thus removed, the sample was connected to the manometer and the vapor pressure recorded after it remained constant for five minutes. The stopcock was turned to connect the two arms and isolate the sample. The apparatus was then wiped and weighed to determine the amount of chloroform removed.

This procedure of removal of chloroform, determination of vapor pressure, and weighing was repeated in increments until all the chloroform was removed. The experimental data are given in Table I. The sharp break at 72 mm. and 15.5% chloroform indicates that the complex contains a 2:1 mole ratio of chloroform to hexamminecobalt(III) benzylpenicillinate.

Decomposition of Hexamminecobalt(III) Benzylpenicillinate Trihydrate into Known Derivatives.—Hexamminecobalt(III) benzylpenicillinate trihydrate (1 g.) was dissolved in 20 ml. of water and 20 ml. of diethyl ether was added. With shaking, the mixture was acidified dropwise with 1:10 concentrated hydrochloric acid:water until no more ether-soluble precipitate was formed momentarily in the aqueous layer. Three to four drops of acid in excess were then added. The layers were separated. The aqueous layer had *p*H 2.1. The colorless organic layer was dried ten minutes over 2 g. of anhydrous sodium sulfate and filtered. The sodium sulfate was washed with 80 ml. of diisopropyl ether, and the filtrate and washings were combined. Almost immediate precipitation occurred, and, after forty-five minutes in the refrigerator, the white crystals were filtered off and washed with 50 ml. of diisopropyl ether in three portions. The dried product, benzylpenicillinic acid diisopropyl etherate, weighed 1.0 g. (93%), assayed 1408 units/mg. (theoretical, 1361 units/mg.), and was analyzed by the N-ethylpiperidine method for 95.8% benzylpenicillin. This product was identical in every respect to an authentic sample prepared by the procedure of Trenner and Buhs.[4]

The acidified aqueous layer from the above preparation was brought to neutrality with dilute aqueous potassium hydroxide, and an excess of aqueous potassium oxalate was added. Precipitation occurred in about five seconds leaving a colorless mother liquor. The filtered, water-washed, and dried precipitate was identical in every respect to an authentic sample of hexamminecobalt(III) oxalate.

TABLE I

DETERMINATION OF THE VAPOR PRESSURE AND COMPOSITION OF THE HEXAMMINECOBALT BENZYLPENICILLINATE–CHLOROFORM COMPLEX

Sample, g.	Weight per cent. chloroform	Vapor pressure, mm.
	Run I	
2.3811	69.6	239
1.9512	62.9	240
1.6631	56.4	237
1.4671	50.6	236
1.3402	45.9	235
1.2127	40.2	233
1.0980	34.0	219
.9864	26.5	196
.8579	15.5	72
.8144	11.0	70
.7841	7.6	67
.7566	4.2	66
.7367	1.6	57
.7269	0.3	27
.7249	0.0	4
	Run II	
2.2650	72.5	225
1.1231	44.6	225
.7764	19.9	156
.7624	18.4	147
.7371	15.6	68
.6702	7.2	62
.6219	0.0	4

Summary

Chloroform reacts with potassium benzylpenicillinate and hexamminecobalt(III) chloride in aqueous solution to give a slightly soluble crystalline complex with a vapor pressure of 70 mm. at room temperature. This loses chloroform on standing in air to form hexamminecobalt(III) benzylpenicillinate, a stable salt wherein the cobalt moiety has no effect on the activity of the penicillin moiety.

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(3) Bjerrum and McReynolds, "Inorganic Syntheses," Vol. II, McGraw-Hill Book Co., Inc., New York, N. Y., 1946, pp. 217–218.

(4) Trenner and Buhs, THIS JOURNAL, **70**, 2897 (1948).