vapor pressure measurements, following a standard method,[4] always made on the day of final purification, employed mercury thermometers which had been calibrated in the same apparatus with boiling water, bromobenzene or aniline; the apparatus was of Pyrex glass, and included stopcocks and ground joints in non-critical positions. Errors in temperatures could not have exceeded 0.3°; the four vapor pressure equations predicted the observed points with an average error of approximately three mm. All three methyl silicon isocyanates showed sharp melting points, and freezing points without perceptible supercooling; on the other hand, *n*-butyl silicon tended to supercool and form a glass to such an extent that the use of liquid nitrogen and vigorous stirring were necessary to obtain a crystalline solid of fairly sharp melting point. Due to incomplete drainage and other uncertainties, the readings of the toluene thermometer used in obtaining some of the melting points appeared subject to errors possibly as great as three degrees. Densities were obtained using a special 2-ml. micropycnometer.

Discussion

Swarts' rule of linear progression in boiling points[5] does not fit the new methyl compounds satisfactorily. Calculations based on the "increment method," by adding 32.0° to the boiling point of the corresponding chloride[6] for each replacement of one chlorine by isocyanate, predicted the following boiling points: monoisocyanate, 89.7° (57.7 + 32.0); diisocyanate, 134.0° (70.0 + 64.0); triisocyanate, 161.7° (65.7 + 96.0).

After this isolation of the complete series of methyl silicon isocyanates, and of *n*-butyl silicon triisocyanate, the stable existence of at least a part of the ethyl and *n*-propyl series can be inferred.

Summary

1. Certain alkylchlorosilanes yielded the corresponding (new) isocyanates upon treatment with silver isocyanate.

The complete methyl series consisted of trimethyl silicon isocyanate, $(CH_3)_3Si(NCO)$, boiling at 91.0°, dimethyl silicon diisocyanate, $(CH_3)_2Si(NCO)_2$, boiling at 139.2°, and methyl silicon triisocyanate, $(CH_3)Si(NCO)_3$, boiling at 170.8°.

n-Butyl silicon triisocyanate, $(n\text{-}C_4H_9)Si(NCO)_3$, boiling at 215.5°, showed a marked tendency to supercool.

2. Physical data included vapor pressure equations, melting points, refractive indices, densities and molar refractions. The heat of hydrolysis seems to decrease as the number of alkyl groups increases.

(4) See for instance: Mack and France, "Laboratory Manual of Physical Chemistry," D. Van Nostrand Co., Inc., New York, N. Y., 1934, p. 47.

(5) Swarts, *Bull. soc. chim.*, **35**, 1557 (1924).

(6) Anderson, THIS JOURNAL, **64**, 1757 (1942).

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[CONTRIBUTION FROM RESEARCH LABORATORIES, THE UPJOHN COMPANY]

Actidione, an Antibiotic from Streptomyces Griseus[1]

BY JARED H. FORD AND BYRON E. LEACH

It has been found in this Laboratory that the beers from streptomycin-producing strains of *Streptomyces griseus* contain another antibiotic which we have named actidione. In contrast to streptomycin, actidione is very effective against many yeasts but has little or no activity against bacteria.

Simultaneous bio-assays for actidione and streptomycin on a series of production beers demonstrated a wide variation in the ratio of concentrations of the two antibiotics. In a few cases the actidione content was as high as 200-250 mg. per l. but the average was about 80–100 mg. per l. A number of strains of *S. griseus* which produce actidione but little or no streptomycin and vice versa have been obtained by irradiating the conidia with X-rays.[2]

The crude product was isolated from the filtered beer by extracting with chloroform either directly or after a preliminary concentration step which involved adsorption on activated carbon, elution with 80% acetone and removal of acetone from the eluates by distillation. The chloroform extracts were orange-brown or an intense green, depending on the culture medium employed, but most of the color was removed by treatment with carbon. After removing the chloroform by distillation *in vacuo*, the resulting crude products were orange-brown sticky oils having a moldy odor. Bio-assays indicated a purity of 30–60% based on crystalline actidione as the standard.[3]

(1) An outline of this work was reported in a preliminary communication: Leach, Ford and Whiffen, THIS JOURNAL, **69**, 474 (1947).

(2) Observations of Drs. George M. Savage and Alma J. Whiffen of these Laboratories.

(3) The assay method was developed by Dr. Alma J. Whiffen. It is a modification of the paper-disc plate method for streptomycin[4] and employs *Saccharomyces pastorianus* ATCC 2366 as the test organism. The medium consists of 10 g. of glucose, 2.5 g. of Difco yeast extract, 1.0 g. of potassium dihydrogen phosphate and 20 g. of agar made up to 1.0 l. with distilled water and adjusted to *p*H 6.0. The inoculation was at the rate of 1.8×10^6 yeast cells per ml. of medium. The *p*H of the aqueous test solutions is not critical and the samples may also be assayed in methanol, ethanol or acetone.

(4) Loo, Skell, Thornberry, Ehrlich, McGuire, Savage and Sylvester, *J. Bact.*, **52**, 610 (1945).

TABLE I

ANALYTICAL DATA

Compound	Formula	Calculated for C_{15} Formula C	H	N	Calculated for C_{27} Formula C	H	N	Found[c] C	H	N
Actidione	$C_{15}H_{23}NO_4$ or $C_{27}H_{42}N_2O_7$	64.02	8.24	4.98	64.01	8.36	5.53	64.16	8.17	5.13
Acetate	$C_{17}H_{25}NO_5$ or $C_{31}H_{46}N_2O_9$	63.14	7.79	4.33	63.03	7.85	4.74	63.13	7.53	4.47
Oxime	$C_{15}H_{24}N_2O_4$ or $C_{27}H_{44}N_4O_7$	60.79	8.16	9.46	60.42	8.27	10.44	60.66[b]	8.29[b]	9.62
Semicarbazone hydrate[a]	$C_{16}H_{28}N_4O_5$ or $C_{27}H_{52}N_8O_9$	53.92	7.92	15.72	53.03	7.98	17.06	53.73[b]	8.16[b]	16.19[b]

[a] Calculated as a monohydrate for the C_{15} formula and as a dihydrate for the C_{27} formula. [b] Analysis by Oakwold Laboratories. Other analyses by our Analytical Department under the supervision of Mr. Harold Emerson. [c] Averages of two or more determinations. Individual results given in Experimental.

These crude products proved to be highly irritating to the skin.

Crystalline material was first obtained by counter-current distribution[5] between benzene and water, using separatory funnels. A comparison of the experimental curve with the theoretical curve (Fig. 1) would indicate that the central band was composed, in the main part, of one material. Since the activity was also located in this band, it can be concluded that the material in this band is primarily actidione. The emulsions that were encountered by this method proved to be troublesome. For the isolation of larger amounts of crystalline material carbon chromatography was found to be more convenient. The crude product was put on the column in 20% acetone, developed with 20% acetone and eluted from the column with 60–100% acetone. The peak eluate fractions gave solids that could be crystallized readily by adding amyl acetate and seeding. Several recrystallizations from amyl acetate gave a constant melting point of 115–116.5°. Further recrystallization from water or 30% methanol gave a melting point of 115–117°. The specific rotation is −3.0° in methanol (c = 10) and +6.8° in water (c = 2). The crystalline product is very soluble in all the common organic solvents with the exception of the saturated hydrocarbons. At 2° the solubility is 2.1 g. per 100 ml. in water and 7 g. per 100 ml. in amyl acetate.

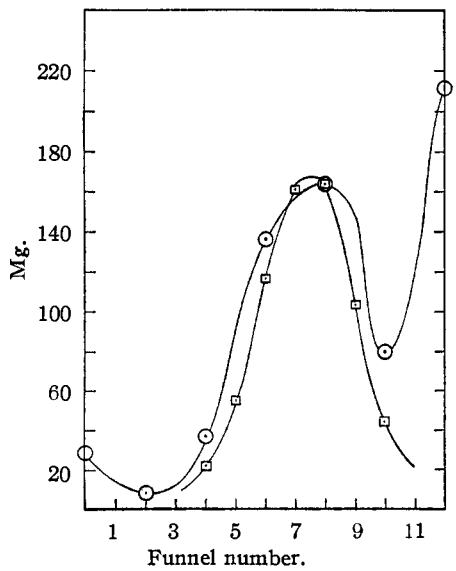

Fig. 1.—⊙, experimental; ⊡, theoretical.

Treatment of the compound with acetic anhydride and pyridine at room temperature produced a biologically inactive acetate, m.p. 148–149°. The oxime, m.p. 203–204°, was also inactive but the semicarbazone, m.p. 182–183°, was found to be about one-twentieth as active as actidione. The melting points of the acetate and semicarbazone are somewhat higher than those reported previously.[1]

Analytical data for carbon, hydrogen and nitrogen on actidione and its derivatives are listed in Table I. It will be noted that the majority of the data is in better agreement with a $C_{15}H_{23}NO_4$ formula for actidione[6] than for our previously suggested $C_{27}H_{42}N_2O_7$ formula. The only values which differ greatly enough to be of much use in distinguishing between the two formulas are the carbon percentages of the semicarbazones and nitrogen contents of the oximes and semicarbazones.

Our previous cryoscopic molecular weight data, using benzene as the solvent, were inconclusive, apparently because of association. The values ranged from 420 to 875, depending upon the concentration. We have now obtained satisfactory molecular weight determinations on actidione and its oxime by the Rast method and on the acetate by a cryoscopic determination in benzophenone (the acetate decomposed at the melting point of camphor). The results (listed in Table II) are in good agreement with the C_{15} formula and appear to exclude any C_{27} or C_{30} formula.

TABLE II

CRYOSCOPIC MOLECULAR WEIGHT DETERMINATIONS

Compound	Formula	Solvent	Molecular weight Calculated	Found
Actidione	$C_{15}H_{23}NO_4$	Camphor	281	254
Acetate	$C_{17}H_{25}NO_5$	Benzophenone	323	334
Oxime	$C_{15}H_{24}N_2O_4$	Camphor	296	291

(5) Craig, Golumbic, Mighton and Titus, *J. Biol. Chem.*, **161**, 321 (1945).

(6) This formula was first suggested by Dr. Reuben G. Jones of the Lilly Research Laboratories, Indianapolis, Indiana.

Degradation studies on actidione are being carried out by Drs. Reuben G. Jones and Edmund C. Kornfeld of The Lilly Research Laboratories.

The acute toxicity of actidione has been found to vary widely with the species of test animals employed. In addition to our previously reported value of 150 mg. per kg. for the LD_{50}, intravenously in mice, the following results have been obtained: subcutaneously in guinea pigs, 60 mg. per kg.; intravenously in rabbits, 17 mg. per kg.; intraperitoneally in cats, 4 mg. per kg.; subcutaneously in rats, 2.7 mg. per kg.; intravenously in rats, 2.5 mg. per kg.

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Experimental

Isolation of Crude Actidione.—Eleven hundred liters of seventy-two hour *S. griseus* beer which assayed 110 mg. of actidione per liter was acidified[7] with sulfuric acid to *p*H 2 and stirred with a mixture of 2 kg. of carbon (Nuchar C-190NU) and 5 kg. of Celite #545[8] and then filtered. The press cake was eluted successively with 40 l. of acetone, three 30-l. volumes of acetone and 20 l. of water. The combined eluates were concentrated *in vacuo* to 38 l. and extracted with 5 l. of chloroform followed by three extractions with 3 l. volumes of chloroform. The combined extracts were decolorized with 300 g. of carbon (Nuchar C-190NU), concentrated *in vacuo* to a sirup, and warmed to 30° under 200–500 μ pressure overnight to remove chloroform. The resulting very thick sirup weighed 97.5 g.; purity, as indicated by bioassay, 59%; 48% yield of activity from beer to crude product.

Counter-Current Distribution Studies on Crude Actidione.—Preliminary tests indicated that crude actidione could be distributed between benzene and water with a partition coefficient of approximately 0.70. Nine-hundred and eighty milligrams of crude actidione was dissolved in 40 ml. of benzene and equilibrated with an equal volume of water in separatory funnels. Twelve such transfers were carried out using the aqueous layer as the traveling phase according to the method described by Craig, Golumbic, Mighton and Titus.[4] The emulsions that were encountered during these transfers were broken by centrifugation. Figure 1 shows the weight curve obtained from this 12-transfer distribution. The biologically active fractions very closely approximated the peak fractions (nos. 6, 7, 8 and 9) and these were combined and dried. One hundred and twenty-seven milligrams of this dried product was dissolved in 4 ml. of anhydrous ethyl ether and poured into 150 ml. of low boiling petroleum ether. The milky suspension crystallized slowly after vigorous scratching with a glass rod. After standing overnight in the refrigerator the crystals were collected and dried; weight 43 mg. Recrystallization from ether–petroleum ether gave crystals melting at 112–115°.

Carbon Chromatography.—Ten grams of crude actidione (bio-assay, 53% actidione) was dissolved in sufficient acetone to give 80 ml. of solution. The acetone solution was diluted with 320 ml. of water and poured into a 4-foot length of 2-inch Pyrex pipe containing a mixture of 100 g. of Darco G-60 and 100 g. of Celite that had been slurried with 20% acetone and packed under pressure. Using a flow rate[9] of 12 ml. per minute the column was developed with 1500 ml. of 20% acetone followed by 500 ml. of 60% acetone, 500 ml. of 80% acetone, 500 ml. of 90% acetone and 500 ml. of 100% acetone. The eluates were collected in 100-ml. volumes. The first 2000 ml. contained a total of 0.92 g. of light yellow gummy solids, none of which yielded crystals when treated with amyl acetate (2 ml. per g.) and seeded. The next three 100-ml. eluates yielded 1.28 g., 1.31 g. and 0.88 g. of white fluffy solids which crystallized readily when treated with amyl acetate. The next six 100-ml. eluates also gave solids which could be crystallized but were rather gummy in appearance. The total recovery of solids was 9.33 g., of which 7.53 g. was in fractions that could be crystallized. The combined weight of crystals was 4.05 g.; m. p. 113–115°.

Preparation of Pure Actidione.—Two recrystallizations from amyl acetate (2 ml. per g.) raised the melting point of the above-described product to 115–116.5° but subsequent recrystallizations from this solvent caused no change. The analytical sample was recrystallized twice from 30% methanol; m. p. 115.5–117°; $[\alpha]^{25}D$ −3.0° (*c* 10, methanol); +6.8° (*c* 2, water).

Anal. Calcd. for $C_{15}H_{23}NO_4$: C, 64.02; H, 8.24; N, 4.98. Found: C, 64.17, 64.02, 64.23, 64.24; H, 8.00, 8.53, 8.25, 7.91; N, 5.17, 5.10.

Actidione Acetate.—One gram of actidione was dissolved in 5 ml. of acetic anhydride by warming on the steam-bath. After the solution had cooled to room temperature 5 ml. of dry pyridine was added. After standing eighteen hours at room temperature the volatile products were removed *in vacuo*. The residue crystallized upon standing. When recrystallized from 99% isopropanol the yield was 0.83 g.; m. p. 143–149°. Two recrystallizations from hot water gave glistening plates; m. p. 148–149°; $[\alpha]^{25}D$ +22° (*c* 2.3, methanol).

Anal. Calcd. for $C_{17}H_{25}NO_5$: C, 63.14; H, 7.79; N, 4.33. Found: C, 63.04, 63.22; H, 7.45, 7.61; N, 4.50, 4.45.

Actidione Oxime.—A solution of 1.0 g. of actidione in 2.5 ml. of methanol was added to a solution of 1.4 g. of hydroxylamine hydrochloride and 2.4 g. of anhydrous sodium acetate in 10 ml. of water. Upon standing overnight at room temperature 0.80 g. of white crystals was obtained; m. p. 194–196° (dec.). The analytical sample was recrystallized from methanol; m. p. 203–204° (dec.).

Anal. Calcd. for $C_{15}H_{24}N_2O_4$: C, 60.79; H, 8.16; N, 9.46. Found: C, 60.79, 60.52; H, 8.26, 8.32; N, 9.65, 9.59.

Actidione Semicarbazone.—A solution of 1.0 g. of actidione in 5 ml. of methanol was added to a solution of 1.0 g. of semicarbazide hydrochloride and 1.3 g. of anhydrous sodium acetate in 5 ml. of water. Upon standing overnight at room temperature 1.13 g. of white crystals was obtained; m. p. 175–178°. Recrystallization from methanol raised the melting point to 182–183°.

Anal. Calcd. for $C_{16}H_{26}N_4O_4 \cdot H_2O$: C, 53.92; H, 7.92; N, 15.72; H_2O, 5.05. Found: C, 53.72, 53.74; H, 7.95, 8.37; N, 16.08, 16.30; H_2O (by drying to constant weight at 60–80° in high vacuum), 5.09.

Summary

1. Actidione appears to have the formula $C_{15}H_{23}NO_4$ rather than $C_{27}H_{42}N_2O_7$ as previously suggested.

2. Methods for its isolation and purification are described.

3. Acute toxicities vary greatly with the species of test animals employed.

KALAMAZOO, MICHIGAN RECEIVED SEPTEMBER 27, 1947

(7) The acidification aids the subsequent filtration of the carbon-mycelium cake. It is unnecessary for adsorption of the actidione.

(8) A filter aid composed of diatomaceous earth.

(9) The rate of flow was regulated by adjustment of the pressure at the top of the column.