room temperature, of IV was found to be 0.01 g./100 ml. while that of VIII was 0.27 g./100 ml.

This is the first instance that we are aware of in which a dihaloglycoluril has been isolated. Because VII was converted almost entirely into VIII before any significant amount of IV was observed, we are led to believe that other glycolurils could be similarly chlorinated. However, because of different solubility characteristics, the partial chlorination of other glycolurils might not be as easy to follow visually as was our example.

Chlorination of the related diureidopentane (IX), prepared by the method of de Haan,[7] gave tetrachlorodiureidopentane (X) but, because of the great insolubility of the materials involved, the chlorination proceeded with greater difficulty.

The products described are relatively stable. Pure, dry samples of IV and VIII have been kept in stoppered clear-glass vials at room temperature for as long as two years with only a 5–10% loss of available chlorine. However, mixtures with wet, strongly alkaline materials (sodium metasilicate and sodium metasilicate pentahydrate) resulted in rapid decomposition of IV and VIII, which, on occasion, became violent.

EXPERIMENTAL[8]

Glycoluril (VII). A stirred solution of 30% aqueous glyoxal (2250 g., 11.6 mole) and urea (1900 g., 31.7 mole) in 4 l. of water was heated to 85–95° and maintained at this temperature for 20–30 min. while concentrated hydrochloric acid (25–45 ml.) was added as needed to maintain the solution at *p*H 1.5–2.0. Cooling, filtering, and recrystallizing from water with the aid of decolorizing carbon gave 850–900 g. (52–55%) of white crystalline VII, decomposing at 300°.

Tetrachloroglycoluril (IV). A stirred suspension of VII (71 g., 0.5 mole) in 3200 ml. of water was treated with chlorine (150 g., 2.1 mole) at the rate of 20–40 g./hr. while 6*N* sodium hydroxide solution was added at such a rate as to maintain the mixture at *p*H 7–8, as measured with a *p*H meter. The resulting white solid was filtered, washed twice with 1-l. portions of water, and dried to give 136 g. (97%) of IV, decomposing slowly above 280°.

Anal. Calcd. for $C_4H_2Cl_4N_4O_2$: C, 17.2; H, 0.7; Cl, 50.7; N, 20.0. Found: C, 17.5; H, 0.8; Cl, 50.5; N, 20.2. Infrared examination did not show the NH band (3170 cm.$^{-1}$) present in VII.

Dichloroglycoluril (VIII). This was carried out as in the preparation of IV except that 78 g. (1.1 mole) of chlorine was used. The solution was filtered to remove traces of IV and concentrated under vacuum at 50° to a volume of about 200 ml. The resulting solid was filtered, washed with two 100-ml. portions of water, and dried to give 90 g. (85%) of VIII, melting with rapid decomposition at 180°.

Anal. Calcd. for $C_4H_4Cl_2N_4O_2$: C, 22.8; H, 1.9; Cl, 33.6; N, 26.5. Found: C, 22.5; H, 1.6; Cl, 33.0; N, 26.0.

Tetrabromoglycoluril (V). A stirred suspension of VII (7.1 g., 0.05 mole) in 2200 ml. of water was treated with bromine (80.0 g., 0.5 mole) over a 3-hr. period while the mixture was maintained at *p*H 9–10. The resulting solid after filtering, washing with two 500-ml. portions of water, and drying gave 17.2 g. (75%) of V melting at 292–295° with decomposition.

Anal. Calcd. for $C_4H_2Br_4N_4O_2$: C, 9.8; H, 0.4; Br, 69.6. Found: C, 10.5; H, 0.8; Br, 65.5.

Tetrachlorodiureidopentane (X). A stirred suspension of IX (56 g., 0.3 mole) in 3 l. of water was treated with chlorine (110 g., 1.55 mole) over a 4-hr. period while the mixture was maintained at *p*H 5–8. The white solid was filtered, washed with several portions of water and dried to give 87 g. (90%) of X melting at 210° with decomposition.

Anal. Calcd. for $C_7H_8Cl_4N_4O_2$: Cl, 44. Found: Cl, 41.5.

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(7) T. de Haan, *Rec. trav. chim.*, **27**, 162 (1908).

(8) All melting points are uncorrected. Elemental and infrared analysis by the Diamond Alkali Company Research Analytical Laboratory.

C-73: A Metabolic Product of *Streptomyces albulus*

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Received August 11, 1959

C-73 is a crystalline compound which accompanies cycloheximide and E-73 in the broths of *Streptomyces albulus*. The three compounds have identical carbon skeletons. C-73 has an aromatic ring in place of the cyclohexanone ring which is common to cycloheximide and E-73. The structure of C-73 is shown (I.)

The isolation of the five fractions designated as A-73 (fungicidin), B-73, C-73, D-73 (cycloheximide), and E-73 from the culture filtrates of *Streptomyces albulus* has been described earlier.[1] Among these, E-73 showed pronounced antitumor activity in experimental animals and its structure has been elucidated.[2] The present paper deals with the chemical nature of C-73.

C-73 (I) is a pale yellow crystalline solid sparingly soluble in common organic solvents. Elementary analysis corresponds to the empirical formula $C_{15}H_{17}O_4N$. Its occurrence with cycloheximide in the culture broths and the close similarity between their empirical formulae $C_{15}H_{17}O_4N$ and $C_{15}H_{23}O_4N$ suggested a possible structural relationship between the two.

The ultraviolet spectrum of C-73 has maxima at 262 and 345 mμ (ϵ = 10,870 and 4,550 respectively). The infrared spectrum shows bands at 5.80, 5.90, 6.10, and 6.26 μ among others. The substance shows bright yellow fluorescence under ultraviolet light. It gives a dark green color with alcoholic ferric chloride, indicating the presence of a phenolic group. C-73 is soluble in aqueous alkali to give bright yellow solutions.

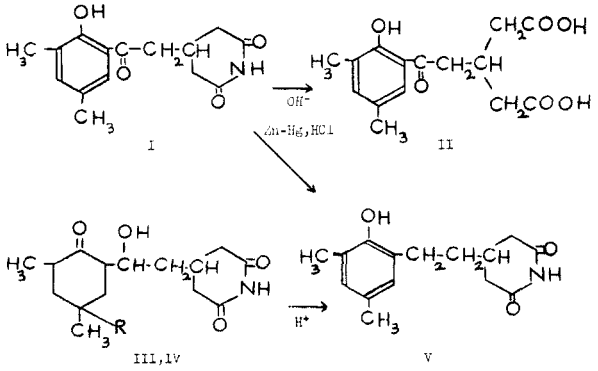
(1) K. V. Rao and W. P. Cullen, *J. Am. Chem. Soc.*, in press.

(2) K. V. Rao, *J. Am. Chem. Soc.*, in press.

Acetylation of C-73 yields a colorless mono acetate, $C_{17}H_{19}O_5N$. C-73 forms an orange red 2,4-dinitrophenylhydrazone as evidence for the presence of the carbonyl group. The color reaction with ferric chloride, the ultraviolet spectrum and the diminished hydroxyl band in the infrared spectrum suggest that the carbonyl group is *ortho* to the phenolic hydroxyl. Boiling C-73 with aqueous alkali produces one molar equivalent of ammonia and an acidic compound (II). This acid, which is dibasic (N = 147) has the molecular formula $C_{15}H_{18}O_6$. It has ultraviolet absorption maxima at 262 and 345 mμ (ϵ = 11,000 and 4600 respectively, similar to the original compound). It also retains the fluorescence and the ferric chloride reaction typical of C-73.

Methylation of C-73 yields a colorless methylation product $C_{17}H_{21}O_4N$ which contains one methoxyl and one methylimide group.

The properties described thus far indicate the presence of a phenolic group, a keto group and an imide group in C-73. It may be recalled that both cycloheximide (III) and E-73 (IV) contain an imide group. As C-73 differs from cycloheximide only by the lack of six hydrogen atoms, the possibility appeared that the former is an aromatized analogue of cycloheximide. Among the alternatives considered, structure I appeared most probable. During the course of the work on the structure of E-73, some of the phenolic transformation products of the latter became available and it appeared that C-73 could be related to one of them. Accordingly C-73 was reduced by the Clemmensen procedure whereby a colorless crystalline product (V) was obtained. This was shown to be identical in all respects to desacetyl dehydro E-73 described earlier.[2] The formation of this common intermediate is considered as a proof for structure I for C-73. The reactions are described in Fig. 1. Unlike cycloheximide or E-73, C-73 has little or no antitumor activity in experimental animals.



III. R = H
IV. R = O Ac

Fig. 1. Comparaison of C-73 with cycloheximide and E-73

EXPERIMENTAL

C-73 was purified by crystallization from a mixture of methanol and chloroform. The product separated out as pale yellow needles, m.p. 198–199°.

Anal. Calcd. for $C_{15}H_{17}O_4N$: C, 65.44; H, 6.22, N, 5.09. Found: C, 65.57; H, 6.33; N, 5.10.

For acetylation, C-73 (0.2 g.) was left at room temperature with acetic anhydride (2 ml.) and pyridine (0.5 ml.) for 24 hr. The reagents were removed by a current of air and the residue crystallized from a mixture of methylene chloride and ether. The acetyl derivative separated as colorless needles, m.p. 149–150°.

Anal. Calcd. for $C_{17}H_{19}O_5N$: C, 64.34; H, 6.04; N, 4.41. Found: C, 63.34; H, 6.52; N, 4.42.

The 2,4-dinitrophenylhydrazone of C-73 was prepared by the action of 2,4-dinitrophenylhydrazine in 2*N* methanolic hydrochloric acid. The derivative separated as orange red rectangular plates which did not melt below 280°.

Anal. Calcd. for $C_{21}H_{21}O_7N_5$: C, 55.38; H, 4.65; N, 15.38. Found: C, 55.84; H, 4.84; N, 15.00.

Alkaline hydrolysis of C-73. A solution of C-73 (0.5 g.) in aqueous sodium hydroxide (25 ml.) was refluxed for 2 hr. A current of nitrogen was passed through the solution during the hydrolysis and the exit gases trapped in 1*N* hydrochloric acid. The distillate was concentrated to dryness and the residue crystallized from methanol-acetone.

Anal. Calcd. for NH_4Cl: N, 26.17; Cl, 66.28. Found: N, 26.85; Cl, 66.10.

The alkaline hydrolysis mixture was acidified and the precipitated solid crystallized from aqueous methanol. The product separated as long, colorless needles, m.p. 126–127°.

Anal. Calcd. for $C_{15}H_{18}O_6$: C, 61.23; H, 6.16. Found: C, 61.16; H, 6.20.

Methylation of C-73. A mixture of C-73 (0.5 g.), acetone (50 ml.), dimethyl sulfate (2 ml.), and anhydrous potassium carbonate (8 g.) was refluxed for 12 hr., the solvent was distilled, the residue treated with water and the mixture extracted twice with methylene chloride. Concentration of the solvent extract gave a colorless crystalline solid which was recrystallized from a mixture of ether-isopropyl ether. The methyl ether separated as colorless rectangular prisms, m.p. 100–101°.

Anal. Calcd. for $C_{17}H_{21}O_4N$: C, 67.32; H, 6.97; N, 4.61; OMe,[1] 10.21; NMe,[1] 9.56. Found: C, 67.46; H, 7.10; N, 4.77; OMe, 10.48; NMe, 8.0.

Reduction of C-73. Zinc amalgam was prepared from zinc dust (5 g.) and a 0.5% solution of mercuric chloride. The supernatant liquid was decanted and a solution of C-73 (0.3 g.) in a mixture of ethanol (20 ml.) and 6*N* hydrochloric acid (20 ml.) was added and the whole refluxed for 4 hr. After 2 hr., an additional quantity (5 ml.) of the acid was added. At the end of the reaction, the mixture was filtered, the residue washed with ethanol, and the filtrate concentrated to remove the ethanol. Extraction of the aqueous concentrate with ether followed by evaporation of the extract gave a colorless crystalline solid. When recrystallized from aqueous methanol, V separated as colorless glistening rectangular plates, m.p. 147–148°. A mixed melting point with desacetyl dehydro E-73[2] (V) (obtained by heating E-73 (IV) with 6*N* hydrochloric acid) was undepressed. The ultraviolet spectra (λ_{max} at 280 mμ, ϵ = 2000) and the infrared spectra were identical.

Anal. Calcd. for $C_{15}H_{19}O_3N$: C, 68.94; H, 7.33; N, 5.36. Found: C, 68.43; H, 7.52; N, 5.67.

Acknowledgment. The author is grateful to Dr. R. L. Wagner for analyses.

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