Summary

Both racemates of 3-methyloctahydropyrrocoline have been obtained for the first time. It has been shown that these compounds, rather than quinolizidine, are produced predominantly in the Clemmensen reduction of 3-ketoquinolizidine.

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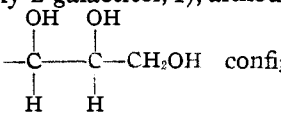
L-Fuco-4-ketose, a New Sugar Produced by the Action of *Acetobacter suboxydans* on L-Fucitol[1]

BY NELSON K. RICHTMYER, LAURA C. STEWART AND C. S. HUDSON

Earlier work from this laboratory by Hann, Tilden and Hudson[1a] has shown that L-fucitol (6-desoxy-L-galactitol, I), although it does not possess the  configuration that they found to be favorable for the ready oxidation of polyhydric alcohols to 2-ketoses by the action of *Acetobacter suboxydans*, was indeed attacked by that organism to a very appreciable extent. The solution resulting from their oxidation of L-fucitol showed a specific rotation of $-7°$. Recently Bollenback and Underkofler[2] have confirmed this biochemical oxidation, and by a reinoculation technique have obtained a 68% oxidation (calculated as glucose) of L-fucitol after twenty days.

The object of our research was to identify the product of this biochemical oxidation of L-fucitol. Under the conditions used, the reaction appeared to be complete by the fifteenth day with a 62% conversion to reducing sugar (calculated as glucose). Deproteinization followed by deionization yielded a solution of the reducing material whose $[\alpha]^{20}D$ value was estimated to be $-5°$. Upon concentration of this solution 13.7 g. of the original 38.7 g. of L-fucitol could be recovered in crystalline form. The remainder of the material was a sirup (20.6 g.) which did not show any inclination to crystallize. With phenylhydrazine it yielded neither L-fucose phenylhydrazone nor phenylosazone, from which we concluded that the principal component was neither L-fucose nor L-fuculose (II); furthermore the rotation of L-fuculose[3] is positive, whereas our product was levorotatory. Hence the new and unknown sugar must have been formed through oxidation of a secondary hydroxyl group on carbon 3, 4 or 5.

To distinguish among these three possibilities the sirup was hydrogenated in the presence of Raney nickel. Each of the three possible carbonyl compounds would be expected to yield L-fucitol and in addition an isomeric alcohol whose identification would then enable us to recognize the parent ketose. Thus if the CHOH group on carbon 3 were oxidized with the formation of compound III, the latter on subsequent reduction should furnish L-fucitol (I) and 6-desoxy-L-gulitol (VI). This last substance, known from the work of Müller and Reichstein[4] and of Bollenback and Underkofler,[2] melts at 133–134° and crystallizes readily. However, after removal of the L-fucitol the inoculation of our residual sirup under favorable conditions with an authentic sample of 6-desoxy-L-gulitol failed to induce crystallization. Formula III was thereby eliminated from further consideration.

Formula V was next discarded when our sirup failed to crystallize when inoculated with 6-desoxy-D-altritol (VIII), a new substance which we prepared especially for this purpose from the methyl 2,3,4-tribenzoyl-6-desoxy-α-D-altroside described in a recent paper from this laboratory.[5] Confirmatory evidence that the second alcohol was not 6-desoxy-D-altritol was found in the latter's levorotation, $[\alpha]^{20}D$ $-9.4°$ in water, whereas the unknown sirupy alcohol showed dextrorotation, $[\alpha]^{20}D$ $+5.4°$.

That left formula IV as the remaining possibility for the new sugar. Upon hydrogenation of a ketose of this structure there should be obtained as the second alcohol 6-desoxy-L-glucitol (VII), usually called L-epirhamnitol. This substance had been prepared as a sirup by Votoček and Mikšič,[6] and its rotation reported as $[\alpha]^{20}D$ $+9.18°$ in water. Although those authors had prepared a crystalline dibenzylidene derivative, we chose to complete the identification of our presumed L-epirhamnitol (VII) by condensing it with formaldehyde in the presence of concentrated hydrochloric acid. Ness, Hann and Hudson[7] had proved that the product obtained in this way from the enantiomorphous alcohol was 1,3:2,4-dimethyl-

(1) Presented in part before the Division of Sugar Chemistry and Technology at the Detroit meeting of the American Chemical Society, April 18, 1950.

(1a) R. M. Hann, E. B. Tilden and C. S. Hudson, THIS JOURNAL, **60**, 1201 (1938).

(2) G. N. Bollenback and L. A. Underkofler, *ibid.*, **72**, 741 (1950).

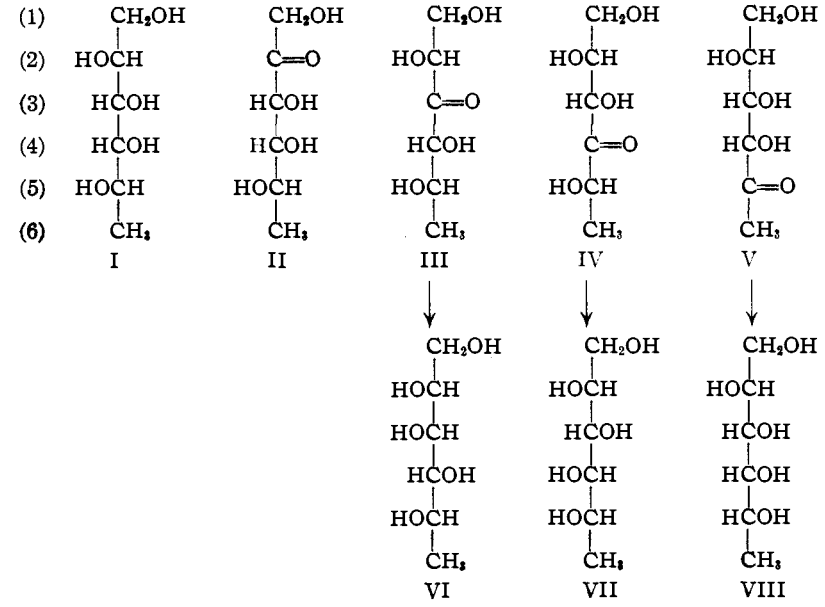
(3) J. Barnett and T. Reichstein, *Helv. Chim. Acta*, **20**, 1529 (1937); **21**, 913 (1938).

(4) H. Müller and T. Reichstein, *ibid.*, **21**, 251 (1938).

(5) D. A. Rosenfeld, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **70**, 2201 (1948).

(6) E. Votoček and J. Mikšič, *Bull. soc. chim. France*, [4] **43**, 220 (1928).

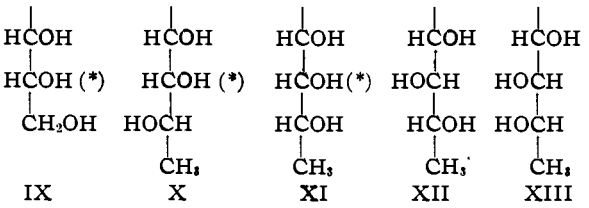
(7) A. T. Ness, R. M. Hann and C. S. Hudson, THIS JOURNAL, **66**, 1235 (1944).



ene-D-epirhamnitol of melting point 182–183° and $[\alpha]^{20}D$ −40.6° in water; the compound we obtained was apparently not quite so pure, as shown by its melting point 178–181° and $[\alpha]^{20}D$ +38.5°. Acetolysis of the dimethylene derivative yielded a triacetate melting at 115–116° and with $[\alpha]^{20}D$ −6.3° in chloroform, values which showed it to be the optical antipode of the previously described 1,5-diacetyl-2,4-methylene-3-acetoxymethyl-D-epirhamnitol[7] melting at 116–117° and with $[\alpha]^{20}D$ +5.3°. Finally, deacetylation produced a monomethylene derivative melting at 176–177° and with $[\alpha]^{20}D$ +19.7° in water, in excellent agreement with the values to be expected from those of the known antipodal 2,4-methylene-D-epirhamnitol[7] melting at 176–177° and with $[\alpha]^{20}D$ −20.2°. Our sirupy alcohol was thus proved to be L-epirhamnitol (VII), and the parent ketose produced by the action of *Acetobacter suboxydans* must have been, for the major part at least, the previously unknown ketose IV.

To the compound IV we give the trivial name L-fuco-4-ketose, which is also sufficiently definite to enable one to infer its configurational formula. Synonymous designations are, for example, 4-keto-6-desoxy-L-galactitol and 1-desoxy-3-keto-D-galactitol, but we prefer a name which carries the -ose ending. Although the sugar has so far remained sirupy, we hope to investigate further the properties and derivatives of what now appears to be the first authentic example of a ketose other than a 2-ketose.

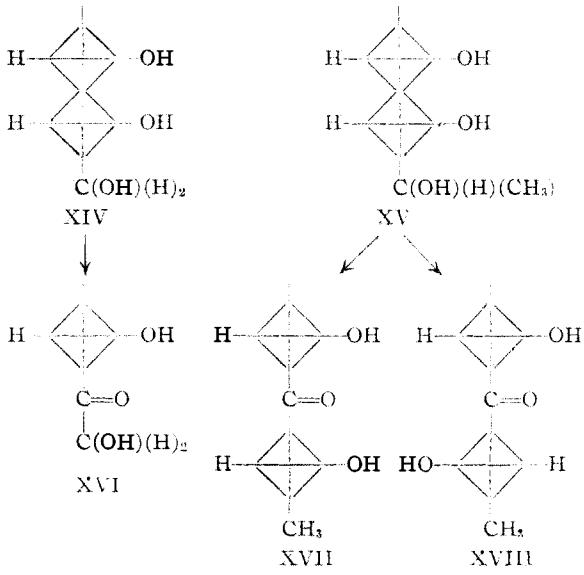
As mentioned earlier, we know from the work of Hann, Tilden and Hudson,[1a] and verification through subsequent examples from this and other laboratories, that only the configuration IX is favorable for the action of *Acetobacter suboxydans* on the polyhydric alcohols containing a terminal CH_2OH group; the point of oxidation is indicated on the formula. If we consider that the formulation X that occurs in L-fucitol is simply IX in which an H has been replaced by a CH_3, that is, that CH_3CHOH is a slightly elongated CH_2OH group, then we might expect that a compound containing the moiety X would also be oxidized at the point indicated. This has now been proved to be true with L-fucitol. Two additional examples of ω-desoxy sugar alcohols with the same configuration as in X have also been found to be oxidized to an appreciable extent (calculated as glucose), namely, 5-desoxy-L-lyxitol (25% in twenty-one days) by Bollenback and Underkofler,[2] and 1,6-dides-oxydulcitol (77% in ten days) in this laboratory. By the same reasoning we should expect that alcohols with the configuration XI also would be oxidized, and indeed two of this type are known to be attacked: 6-desoxy-D-allitol[2] (21% in ten days) and 6-desoxy-D-altritol (VIII) (95% in ten days). On the other hand, there are six possible combinations containing those three asymmetric carbon atoms besides X and XI that we should not expect to be oxidized by *Acetobacter suboxydans*. Among these types, the only alcohols tested that do not contain also the favorable grouping IX at the other end of the molecule are those having configurations XII and XIII. These are 6-desoxy-D-iditol,[2] 6-desoxy-D-gulitol,[2] 6-desoxy-L-mannitol (L-rhamnitol)[1a,2] and 6-desoxy-L-glucitol (L-epirhamnitol, VII)[2]; they were not oxidized. Examples of most of the four remaining types will probably be tested in the near future in an effort to complete this series, and to verify the new extension of the rule[1a] pertaining to the oxidation of acyclic polyhydric alcohols by *Acetobacter suboxydans*.



Finally, the generalizations that may be made from present rather extensive data on the oxidation of acyclic polyhydric alcohols by *Acetobacter suboxydans* are succinctly expressed in the graphic formulas XIV–XVIII.

Experimental

Oxidation of L-Fucitol by *Acetobacter suboxydans*.— As in our preceding study of the oxidation of volemi-



tol,[8] preliminary experiments indicated the adoption of the following procedure. Two liters of medium was prepared to contain 2% of L-fucitol, 0.5% of Difco yeast extract, 0.3% of potassium dihydrogen phosphate and 0.05% of D-glucose. The solution was distributed in 200-ml. portions among ten 2-liter Erlenmeyer flasks and, after sterilization, each flask was inoculated with 1 ml. of a two-day-old culture of *A. suboxydans*[9] grown on a yeast extract medium containing 2% of D-glucose. The mixture was incubated at 30°, with 1-ml. aliquots being removed at intervals, deproteinized and the reducing sugar content determined as described previously. The values, calculated as glucose, were found to be 11.69, 12.46 and 12.37 mg. per ml. at the end of twelve, fifteen and eighteen days, respectively. The culture medium equivalent to 38.8 g. of the original L-fucitol was deproteinized by adding 250 ml. of a solution containing 20% of zinc sulfate heptahydrate followed by enough aqueous barium hydroxide to bring the mixture to *p*H 6.8 (brom thymol blue). The filtered solution was deionized by passage through columns of Amberlite IR-100 and IR-4B and concentrated *in vacuo* to about 1 liter. At this point, on the assumption that the L-fucitol had been 60% oxidized and that any rotation was due entirely to the presence of an unknown sugar, the observed rotation of −0.48° permitted the calculation of an estimated $[\alpha]^{20}D$ −5° for the unknown sugar which was later named L-fuco-4-ketose.

The solution was then concentrated to a thick sirup which upon manipulation with methanol yielded a total of 13.7 g. of unchanged L-fucitol, m. p. 154–156° alone and when mixed with original starting material. The mother liquor was concentrated again to a dry sirup weighing 20.6 g. A 1.2-g. sample of this residue was treated with 2 ml. of phenylhydrazine, 1 ml. of glacial acetic acid and 8 ml. of water in a manner that would be expected to yield L-fucose phenylhydrazone or phenylosazone if L-fucose or L-fuculose had been present. However, the product obtained after heating for one hour on the steam-bath was a red oil which obviously was not L-fucose phenylosazone, and which has not been extensively investigated.

Catalytic Reduction of L-Fuco-4-ketose.—A solution of 18 g. of the sugar sirup was hydrogenated with 6 g. of Raney nickel catalyst for eighteen hours at 100° and 2500 p. s. i. (170 atmospheres). The filtered product was deionized to remove traces of nickel or other inorganic material. The recovered L-fucitol amounted to 5.5 g. and the residual sirup weighed 7.7 g.; some mechanical loss had occurred due to leakage during the hydrogenation. Although the rotation of this residual sirup, $[\alpha]^{20}D$ +4.5° in water, was the same as Bollenback and Underkofler[2] reported for their 6-desoxy-L-gulitol, inoculation of our sirup with crystalline 6-desoxy-L-gulitol prepared by the reductive desulfurization of D-glucose dibenzyl mercaptal failed to induce crystallization. Inoculation with 6-desoxy-L-altritol, whose preparation is described below, was equally unsuccessful. The rotation of our sirup being lower than the value +9.2° reported for sirupy L-epirhamnitol,[6] it seemed probable that the sirup still contained some L-fucitol ($[\alpha]D$ +1.4° in water).[10] The dry sirup was next acetylated with acetic anhydride and fused sodium acetate in the usual manner. The 17 g. of product showed $[\alpha]^{20}D$ −8.4° in chloroform whereas a rotation of −15.7° would be expected for a relatively pure pentaacetyl-L-epirhamnitol[11]; here the presence of pentaacetyl-L-fucitol of $[\alpha]^{25}D$ +20.5° in chloroform[12] would affect considerably the rotation of the pentaacetyl-L-epirhamnitol.

In an attempt to fractionate the presumed mixture of acetates the material was subjected to the technique of flowing chromatography. Adsorption on activated alumina, followed by elution with suitable combinations of hexane, benzene and ether, yielded twelve fractions. The first five fractions of $[\alpha]^{20}D$ about −10° weighed 13 g.; the remainder of the material became progressively less levorotatory, the last two fractions having an $[\alpha]^{20}D$ value of about +1°. Although the separation was not so sharp as desired, some purification was effected because upon deacetylation of the 13-g. portion the rotation of the 5.3 g. of sirup had increased to $[\alpha]^{20}D$ +5.4°.

2,4:3,5-Dimethylene-L-epirhamnitol.—Final proof that the sirup just described contained L-epirhamnitol was secured by dissolving it in 15 ml. each of 37% aqueous formaldehyde and concentrated hydrochloric acid according to Ness, Hann and Hudson,[7] and allowing the solution to evaporate over granular calcium chloride and pellets of potassium hydroxide in an evacuated desiccator at room temperature for several days. The process was then repeated with 10 ml. of the reagents. It now appears that this long-continued action was undesirable for the product was a rather complex mixture. By fractional crystallization from ethanol and from acetone–ether we obtained a small amount of material melting at 240–242°; its analysis indicated it probably was composed of two molecules of the dimethylene-L-epirhamnitol joined through the free hydroxyl group as an acetal to an additional molecule of formaldehyde (*Anal.* Calcd. for $C_{17}H_{28}O_{10}$: C, 52.03; H, 7.19. Found: C, 51.88; H, 7.06). By further fractionation we obtained 0.3 g. of a compound whose m. p. 178–181° and $[\alpha]^{20}D$ +38.5° in water identified it as 2,4:3,5-dimethylene-L-epirhamnitol, the enantiomorph of the derivative of m. p. 182–183° and $[\alpha]^{20}D$ −40.6° previously described by Ness, Hann and Hudson.[7] Because our compound was obviously not pure, its analysis was omitted, but its complete identification was obtained as follows.

1,5-Diacetyl-2,4-methylene-3-acetoxymethyl-L-epirhamnitol.—The acetolysis of 0.5 g. of crude dimethylene-L-epirhamnitol ($[\alpha]^{20}D$ +35.4° in water) by the procedure of Ness, Hann and Hudson[7] yielded 0.52 g. of product which appeared to be pure after one recrystallization from chloroform by the addition of pentane. In order to obtain more acetolysis product all the remaining fractions, consisting of high-melting, low-melting and sirupy material, from the formaldehyde reactions were combined and subjected to acetolysis. Thus the yield of once-recrys-

(8) L. C. Stewart, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **71**, 3532 (1949).

(9) American Type Culture Collection No. 621.

(10) Obtained by reversing the sign of the rotation determined for the enantiomorphous rhodeitol (D-fucitol) by E. Votoček and J. Bulíř, *Z. Zuckerind. Böhmen*, **30**, 333 (1906).

(11) Obtained by converting under similar conditions a sample of sirupy D-epirhamnitol to its sirupy acetate whose rotation was estimated as $[\alpha]^{20}D$ +15.7° in chloroform.

(12) M. L. Wolfrom, W. J. Burke and S. W. Waisbrot, THIS JOURNAL, **61**, 1827 (1939).

tallized 1,5-diacetyl-2,4-methylene-3-acetoxymethyl-L-epirhamnitol was increased to 2.1 g. After two additional recrystallizations the m. p. 115–116° and $[\alpha]^{20}D$ −6.3° in chloroform (*c*, 4) were in good accord with the m. p. 116–117° and $[\alpha]^{20}D$ +5.3° in chloroform (*c*, 0.9) reported for the antipodal D-form.[7]

Anal. Calcd. for $C_{14}H_{22}O_9$: C, 50.29; H, 6.63; CH_3CO, 38.6. Found: C, 50.33; H, 6.83; CH_3CO, 38.8.

2,4-Methylene-L-epirhamnitol.—Deacetylation of 2.0 g. of the preceding compound in 10 ml. of chloroform cooled in an ice-bath was effected catalytically by adding 1 ml. of 3% sodium methoxide. Fine needles appeared spontaneously within an hour and crystallization was allowed to continue for two days in the refrigerator. Filtered and washed with chloroform the granular powder melted at 167–172° and weighed 0.97 g. (91%). Two recrystallizations from 15 parts of 95% alcohol furnished 0.4 g. of small, prismatic needles of 2,4-methylene-L-epirhamnitol, m. p. 176–177° and $[\alpha]^{20}D$ +19.7° in water (*c*, 1.4); Ness, Hann and Hudson[7] recorded m. p. 176–177° and $[\alpha]^{20}D$ −20.2° in water (*c*, 0.9) for the enantiomorph.

Anal. Calcd. for $C_7H_{14}O_5$: C, 47.18; H, 7.92. Found: C, 47.04; H, 7.72.

Preparation of 6-Desoxy-D-altritol from Methyl 2,3,4-Tribenzoyl-6-desoxy-α-D-altroside.—Thirty-eight grams of the pure altroside derivative[5] was debenzoylated catalytically by dissolving it in 400 ml. of hot methanol containing 1 ml. of 3% sodium methoxide and leaving the solution at room temperature for twenty-four hours. Water was added, and the mixture concentrated *in vacuo*, more water being added as necessary, until all the methanol and methyl benzoate had been removed. The sirup obtained by further concentration was dissolved in 400 ml. of 0.5 *N* hydrochloric acid. The rotation of this solution was calculated as $[\alpha]^{20}D$ +111° for methyl 6-desoxy-α-D-altroside, which is near the $[\alpha]^{16}D$ +118.6 ±2° in methanol reported by Gut and Prins[13] for their sirupy methyl 6-desoxy-α-D-altroside. The hydrolysis of this glycoside, which appeared to be nearly complete after boiling the solution for one hour, was continued for five hours. The equilibrium rotation of the 6-desoxy-D-altrose thus formed was estimated as $[\alpha]^{20}D$ +18°, in good agreement with the value +16 ±2° in water recorded by Gut and Prins[13] and in harmony with the −17.3° and −18° found for two samples of the antipodal 6-desoxy-L-altrose prepared by Freudenberg and Raschig[14]; neither sugar has yet been obtained in crystalline form.

The hydrolysis mixture was freed from acid by passage through a column of Duolite A-3, concentrated *in vacuo* and the residue hydrogenated in 100 ml. of water with 3 g. of Raney nickel for seven hours at 100° and 2500 p. s. i. (170 atmospheres). The solution, after filtration, was again deionized with Amberlite IR-120 and Duolite A-3. Upon concentration *in vacuo*, crystals of the 6-desoxy-D-altritol appeared spontaneously. The dry residue was crystallized from methanol as clusters of acicular prisms. The 8.8 g. obtained in the first two crops represented an over-all yield of 68% from the original altroside derivative. Neither the melting point (116–119° to a viscous melt full of bubbles) nor the rotation ($[\alpha]^{20}D$ −9.4° in water; *c*, 4) was changed by two additional recrystallizations. In 5% ammonium molybdate (*c*, 0.40) the 6-desoxy-D-altritol showed $[\alpha]^{20}D$ +113°; in acidified ammonium molybdate solution (20 ml. of 5% ammonium molybdate and 5 ml. of *N* sulfuric acid; *c*, 0.40) the rotation was $[\alpha]^{20}D$ −43.3°.

Oxidation of 6-Desoxy-D-altritol and 1,6-Didesoxydulcitol[15] by *Acetobacter suboxydans*.—Preliminary studies of the biochemical oxidation of these alcohols under the same conditions that were used for L-fucitol indicated their conversion to reducing substances to the extent of 95% and 77%, respectively, calculated as D-glucose, in ten days.

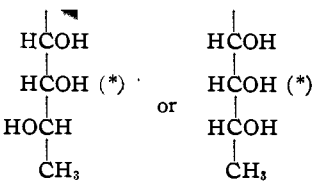
Acknowledgment.—The authors wish to thank Mr. William C. Alford, Mrs. Margaret M. Ledyard and Mrs. Evelyn G. Peake of this Institute for carrying out the microchemical analyses.

Summary

The oxidation of L-fucitol (6-desoxy-L-galactitol) by *Acetobacter suboxydans* has yielded a new sugar to which we have given the trivial name L-fuco-4-ketose. Proof of this structure was secured by hydrogenating the sirupy sugar to a mixture of L-fucitol and L-epirhamnitol (6-desoxy-L-glucitol). Identification of the latter substance was effected by converting it to three crystalline methylene derivatives and comparing them with the corresponding known D-epirhamnitol derivatives.

6-Desoxy-D-altritol has been obtained in crystalline form. Both 6-desoxy-D-altritol and 1,6-didesoxydulcitol are oxidizable by *A. suboxydans*.

All the evidence so far available indicates that only the ω-desoxy sugar alcohols with the configuration



are oxidizable by *A. suboxydans*, and at the point indicated. By considering the CH_3CHOH group as simply an elongated CH_2OH group, the Bertrand rule, as modified by Hann, Tilden and Hudson for *A. suboxydans*, can now be extended to predict the action of that organism upon the ω-desoxy sugar alcohols.

BETHESDA, MARYLAND RECEIVED APRIL 29, 1950

(13) M. Gut and D. A. Prins, *Helv. Chim. Acta*, **29**, 1555 (1946).

(14) K. Freudenberg and K. Raschig, *Ber.*, **62**, 373 (1929).

(15) A. T. Ness, R. M. Hann and C. S. Hudson, THIS JOURNAL, **64**, 982 (1942).