esulting solutions were evaporated to dryness and the salts recrystallized repeatedly from absolute methanol.

The physical and analytical properties of these materials are summarized in Table IV. The quaternary salts were analyzed as the dipicrates, because of the extreme ease of decomposition of the chlorides at elevated temperatures.

TABLE IV

PHYSICAL AND ANALYTICAL CONSTANTS

Compound		IV	V	VI[a]	VII
B.p.	°C.			83–84[b]	84–85
	Mm.			40	25
$n^{25}D$				1.4551	1.4617
M.p., °C.		202–203 dec.	209–210 dec.		
Dipicrate: %					
Carbon	Calcd.	41.04	42.04	67.59[a]	41.04
	Found	41.07	42.16	67.68[a]	41.54
Hydrogen	Calcd.	4.27	4.49	12.70	4.27
	Found	4.22	4.34	12.65	3.92
Nitrogen	Calcd.	18.24		19.71	
	Found	18.24		19.75	

[a] Analyzed as the free base. [b] Lit. value, 92–94° (48 mm.); L. M. Rice, C. H. Grogan and E. E. Reid, THIS JOURNAL, **75**, 2261 (1953).

Enzymatic Rate Determinations.—The hydrolysis reaction of acetylcholine catalyzed by AChE was followed by titration of the liberated acetic acid, at a constant *p*H of 7.4. Triply recrystallized AC was used in all the rate determinations.

For the reaction vessel, a 4-ml. titration cell was fitted with a water jacket through which water held at constant temperature (25.12 ± 0.02°) was circulated, with a small glass capillary rod for mechanical stirring, and with a small pair of standard electrodes for the Beckman *p*H Meter.

In a representative rate run, 2.50 ml. of buffer was pipetted into the cell and brought to temperature. Then in succession were added 0.50 ml. of the diluted enzyme solution, 0.10 ml. of an inhibitor solution whenever used, and finally 0.10 ml. of an acetylcholine chloride solution of sufficient strength to make its initial concentration in the reaction mixture $3.33 \times 10^{-3} M$. Enzyme concentrations were of the order of 2.0×10^{-5} mg. per ml. After approximately 1 to 2 minutes of stirring to come to bath temperature, readings of the volume of 0.01274 *N* base required to keep the *p*H constant at its initial value were begun. Base was added manually from an ultramicroburet dipping into the reaction mixture. Plots of volume of base added *vs.* time were in general quite linear for about the first five or six minutes of reaction, during which time eight to ten experimental points were obtained.

Runs to determine the normal activity of any given dilution of enzyme were made just prior to the use of that dilution for any determination employing an inhibitor. All inhibitor solutions were freshly prepared.

All points in an enzymatic hydrolysis run were corrected for the spontaneous, non-enzymatically-catalyzed hydrolysis of AC. Under the conditions of these experiments, this spontaneous reaction was found to have a first-order rate constant of 1.1×10^{-6} sec.$^{-1}$, which led to rates amounting to only 1 or 2% of the observed total rate of hydrolysis in any given run. Results on duplicate rate determinations indicated that they were reproducible in general to well within 5%. In any given run, the slope of the reaction curve could be determined within about this same degree of precision (5% or better).

Acknowledgment.—We are indebted to Mr. C. W. Coates and Drs. D. Nachmansohn and H. S. Polin for their most generous aid and advice in the procurement of electric eel tissue and its initial extracts.

BETHESDA, MARYLAND

[CONTRIBUTION FROM THE UNIVERSITY OF TEXAS, M. D. ANDERSON HOSPITAL FOR CANCER RESEARCH]

The Optical Rotation of Human Serum Albumin and γ-Globulin[1]

BY B. JIRGENSONS AND S. SIROTZKY

RECEIVED SEPTEMBER 24, 1953

Serum albumin and γ-globulin were isolated by Cohn's method (#10) from normal sera and from sera of patients with confirmed cancer. The dependence of the optical rotation of these proteins on *p*H was studied, and their resistance to the denaturing influence of guanidine hydrochloride was investigated. It was found that the proteins isolated from sera of cancer patients had somewhat lower rotation values than the normal ones. The rotation values of albumin were not constant between the *p*H limits of 4–10, but a flat maximum of levorotation was found at *p*H 5–7.

Introduction

Blood proteins have been the objects of many investigations but in most instances the studies have been confined to distribution patterns and changes in concentration. Only a few authors have tried to compare the properties of the same kind of proteins isolated from various sources.[2–6] The present study was undertaken in order to ascertain whether the proteins isolated from the blood of cancer patients differ from those isolated from the blood of normal (healthy) individuals. If there were some slight differences in the molecular configuration of proteins from normal serum and from "cancer" serum, the differences could be detected most readily by optical rotation measurements. This assumption is based on previous experience in work on denaturation of proteins. The measurement of optical rotation has been found to be a sensitive means to detect denaturation and also a convenient method for protein characterization.[7]

Experience with the optical activity studies on amino acids showed long ago that single data are of little value. The dependence of the specific rotation of amino acids and proteins on *p*H is, however, very important. Almquist and Greenberg[8] initiated the study of optical rotation of proteins as a function of *p*H, and recently data for more proteins was reported.[7] This study has now

(1) This study was supported in part by grants from the American Cancer Society and the National Cancer Institute, National Institutes of Health. The results were presented at the American Chemical Society 124th National Meeting in Chicago, September 7, 1953.

(2) K. O. Pedersen, *Cold Spring Harbor Symp. Quant. Biol.*, **14**, 140 (1950).

(3) A. B. Gutman, *Advances in Protein Chem.*, **4**, 155 (1948).

(4) F. W. Putnam and B. Udin, *J. Biol. Chem.*, **202**, 727 (1953).

(5) F. W. Putnam, THIS JOURNAL, **75**, 2785 (1953).

(6) R. J. Winzler, *Advances in Cancer Res.*, **1**, 503 (1953).

(7) (a) B. Jirgensons, *J. Polymer Sci.*, **5**, 179 (1950); (b) *ibid.*, **6**, 477 (1951); (c) *Arch. Biochem. Biophys.*, **39**, 261 (1952); (d) *ibid.*, **41**, 333 (1952).

(8) H. J. Almquist and D. M. Greenberg, *J. Biol. Chem.*, **105**, 519 (1934).

been extended to human serum albumin and γ-globulin isolated from normal blood and the blood of cancer patients.

In the present study serum albumin and γ-globulin were isolated by Cohn's method (#10) and the rather dilute solutions obtained by this procedure were used in the preliminary measurements. The optical rotation values of the respective proteins were found to be inconsistent; this was due to errors inherent in low readings (0.2–0.3°). All the samples studied thereafter were concentrated by lyophilization, and solutions containing from 0.4 to 2.5% of protein were used. The results obtained with the more concentrated solution were more consistent. They indicate that the optical rotation of albumin obtained from the blood of cancer patients differs significantly from that of normal subjects. These differences were of a greater order of magnitude if the proteins were denatured with guanidine hydrochloride.

Experimental

Isolation of Proteins.—The proteins were isolated from serum by the method of Cohn (#10), *i.e.*, fractionation with alcohol and heavy metal ions at low temperatures.[9] The fraction I + II + III was washed twice according to the suggestion of Lever and associates.[10] All the procedures of precipitation, washing and centrifugation were performed at about −5°. The treatment of the albumin with Nalcite and dialysis were performed in the refrigerator. Proteins from normal serum and those from cancer patients were prepared in exactly the same fashion. The proteins were pure as tested by means of electrophoresis on paper. The purity also was tested by means of optical rotation; samples of albumin and γ-globulin obtained from Dr. Cohn's laboratory (Harvard University) were used as standards for comparison. Specific rotation values of the albumin and globulin isolated in our laboratory from normal sera were identical to those found for the Harvard samples. The lyophilized samples were preserved in the refrigerator, and only freshly prepared solutions were used for polarimetric measurements. The moisture and ash content of the samples were determined, and the specific rotation values were calculated for moisture-and-ash-free protein. The concentration of the proteins in solutions was determined by evaporation, drying at 105–110°, and weighing. All specimens were dried in the same fashion.

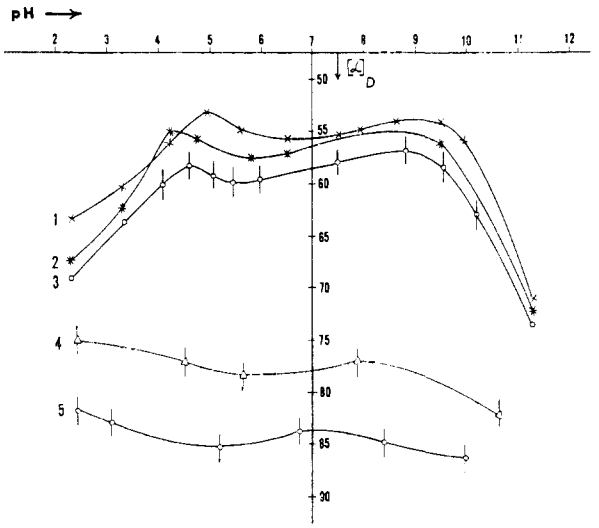

Fig. 1.—The optical rotation of native and denatured serum albumin at various *p*H: 1, native albumin from patients with cancer of prostate; 2, native albumin, cancer of breast; 3, native, normal albumin; 4, albumin from patients with cancer of cervix, denatured with guanidine hydrochloride 2.5 moles/l.; 5, albumin from healthy individuals, denatured with guanidine hydrochloride 2.5 moles/l. The concentration of the albumin is 1%. The vertical lines indicate maximal variations in the 10 to 20 readings of a sample.

Polarimetric Measurements.—The optical rotation was measured with a Schmidt & Haensch precision polarimeter (accuracy ± 0.002°) in a thermostated room at 25°. Semi-micro tubes 10 or 20 cm. long were used. Most of the data reported were obtained with 1.0% solutions of the proteins. The values reported are mean values from 10 to 20 readings, and the variations are indicated by the vertical lines in the figures. Sodium light was used for all determinations.

Dependence of Optical Activity on *p*H.—The *p*H of the protein solutions was varied by means of glycine buffers, the ionic strength in the mixtures being 0.05. Figure 1 shows some of the results obtained with several samples of serum albumin. The saddle-shape of the curves 1–3 is of interest. The optical activity within the *p*H limits of 4–9 is not constant, but instead a maximum of levorotation is evident at about *p*H 5–7. This was not noticed formerly,[8] presumably because of the rather low precision of the polarimetric measurements. The curvature was noticed first on bovine serum albumin,[7c] but the variations were almost in the limits of errors of measurement.

Measurements carried out with our present instrument (which is more sensitive than the one formerly used) gave more conclusive evidence of these changes of specific rotation of serum albumin. These changes near the isoelectric point can be explained as resulting from variations in ionization of the amino and carboxyl groups linked to the asymmetric carbon atoms of the protein molecules. The same effect is known to occur in the case of natural amino acids.[11]

The marked increase in the negative rotation at *p*H's below 4 and above 10 is due to denaturation by acid and alkali, respectively. It is noteworthy that the rotation values for the albumins isolated from sera of cancer patients (curves 1 and 2) are somewhat smaller than those of normal albumin (curve 3), especially at *p*H 5–7.

Figure 2 shows the dependence of specific rotation on *p*H for various specimens of γ-globulin. In this instance the "saddle" in the curves could not be observed. There are two reasons for this: first, the observations near the iso-

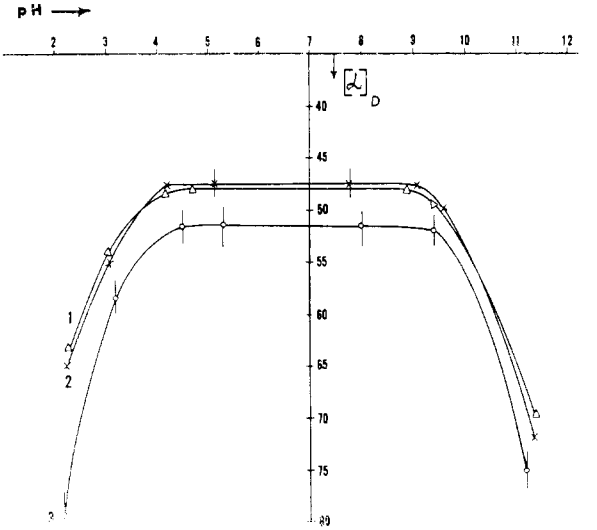

Fig. 2.—The optical rotation of native γ-globulin at various *p*H: 1, globulin from patients with cancer of breast; 2, globulin from patients with cancer of cervix; 3, globulin from normal blood. The concentration of the globulin is 1%.

(9) E. J. Cohn, *et al.*, THIS JOURNAL, **72**, 465 (1950).
(10) W. F. Lever, *et al.*, *J. Clin. Invest.*, **30**, 99 (1951).
(11) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931).

electric point are hampered by turbidity, and, second, the globulin has fewer free amino and carboxyl groups than albumin. Again, the globulins isolated from sera of cancer patients had somewhat lower rotation values (curves 1 and 2) than the normal ones (curve 3). The readings for a pooled sample of "breast cancer" globulin agreed well with those for the globulin from patients with cancer of the cervix uteri. The albumin and γ-globulin differ in that the latter is denatured irreversibly with acid at *p*H's of 2.2–2.5; the rotation values cannot be brought back to the low ones of 48–50° upon neutralizing the solutions.

Denaturation with Guanidine Hydrochloride.—Table I shows the optical activity of serum albumin in the presence of guanidine hydrochloride. The reaction of this denaturing agent increases the specific rotation of the proteins. It seems significant that the albumin isolated from normal serum has considerably larger rotation values than the albumins isolated from sera of cancer patients. Corresponding, but somewhat small, differences were observed between "normal" and "cancer" γ-globulins (Table II).

TABLE I

OPTICAL ROTATION OF ALBUMIN DENATURED WITH GUANIDINE HYDROCHLORIDE; *p*H 4.9–5.2

Albumin	Guanid. HCl, mole/l.	Specific rotation, deg.
Normal, I,[a] 1.0%	2.0	−78.5 ± 1
Normal, II, 0.61%	2.0	−74.6 ± 1.5
Cancer of breast, I, 0.5%	2.0	−68.7 ± 1.5
Cancer of breast, III, 1.0%	2.0	−64.2 ± 1
Cancer of breast, IV, 0.72%	2.0	−64.5 ± 1
Cancer of cervix, I, 2.5%	2.0	−66.3 ± 0.5
Cancer of cervix, II, 1.0%	2.0	−69.4 ± 1
Cancer of cervix, III, 0.84%	2.0	−70.5 ± 1
Normal, 1.0%	2.5	−85.0 ± 1
Cancer of prostate, 0.5%	2.5	−72.2 ± 1.5
Cancer of stomach, 0.65%	2.5	−68.8 ± 1.5
Cancer of breast, I, 0.5%	2.5	−76.8 ± 1.5
Cancer of breast, II, 0.4%	2.5	−73.5 ± 2
Cancer of breast, III, 1.0%	2.5	−77.9 ± 1
Cancer of cervix, IV, 1.0%	2.5	−77.4 ± 1
Cancer of cervix, V, 0.5%	2.5	−74.0 ± 1.5

[a] The Roman numerals refer to different specimens, pooled from 2–3 patients.

TABLE II

OPTICAL ROTATION OF γ-GLOBULIN DENATURED WITH GUANIDINE HYDROCHLORIDE; *p*H 5.2–5.5

γ-Globul	Guanid. HCl, mole/l.	Specific rotation, deg.
Normal, 1.0%	1.25	−53.0 ± 1
Cancer of cervix, 1.0%	1.25	−48.1 ± 1
Normal, 0.5%	1.50	−55.2 ± 1.5
Cancer of cervix, 0.5%	1.50	−49.1 ± 1.5
Cancer of breast, 0.5%	1.50	−52.5 ± 1.5
Cancer of uterus, 0.5%	1.50	−43.8 ± 1.5
Cancer of stomach, 0.94%	1.50	−42.3 ± 1
Normal, 0.5%	2.50	−72.5 ± 1.5
Cancer of uterus, 0.5%	2.50	−62.4 ± 1.5
Cancer of breast, 1.0%	2.50	−70.6 ± 1

Curves 4 and 5 of Fig. 1 show the optical activity of albumin denatured with guanidine hydrochloride (2.5 moles/l.) at various *p*H's. A flat maximum (or minimum or positive rotation) occurs near the isoelectric point, and the same positive shift with increasing acidity and alkalinity is observed as with native albumin. However, there is no increase of rotation at *p*H of 2–4, as the protein is already denatured by the guanidinium salt. There is a small increase of levorotation with time in the presence of guanidine hydrochloride. The data reported are the final, constant values, which were checked after time intervals of several hours, and often after one day.

Discussion

The fact that the proteins isolated from blood of cancer patients have lower optical activities than normal proteins deserves careful critical consideration. The 20 samples of albumin isolated from sera of patients with definite cancer (prostate, breast, cervix, uterus and stomach) had specific rotation values of −51 to −57° (1–2% solutions in water, *p*H 4.9–5.2). The 8 samples of albumin from normal blood, however, had values between −58 and −63°. A sample of crystallized human serum albumin obtained from Harvard University gave −59.8°, and a lyophilized sample from the same institute −61.2°. Cohn and associates[12] reported for human serum albumin an $[\alpha]^{25}_{5461}$ of −78°, and the same figure was reported earlier by Hewitt.[13] Extrapolation according to the linear relationship ascertained by Hewitt, gives for sodium light (wave length 5893 Å.) −60.4°, which is in excellent agreement with our measurements for normal albumin. The most probable average values for normal human albumin are between −59 and −61°, whereas the average values for the albumin from blood of cancer patients lie near −54° (all at *p*H 4.9–5.2).

Since the differences are not great, possible sources of error were considered. First, we excluded the possibility of contamination with other proteins of lower rotations by electrophoresis tests. Second, contaminations with electrolytes of low molecular weights (possibly incomplete dialysis) were excluded by conductivity measurements. Moreover, comparative determinations of sodium with a flame photometer showed that both "normal" and "cancerous" albumin contained the same small concentrations of sodium (about 2.5 millequiv. per 100 ml.).

The differences between the albumins ("cancerous" *versus* "normal") are quite explicit in the solutions containing guanidine hydrochloride (Fig. 1, Table I). This may be interpreted as being the result of varying degrees of stability of the albumins toward the denaturing agent. This is indicated also by the fact that in weakly acidic solutions of *p*H 2.3 the "cancerous" albumins give lower rotation values than the "normal" ones (Fig. 1). Albumins isolated from blood of cancer patients seem to be less reactive than normal albumin; this is in accord with the findings of other authors that the serum proteins of cancer patients have decreased tendency toward coagulation, as well as decreased ability to bind dyes.[6] It is puzzling that the limited data at hand do not show isolated "cancerous" albumin to differ from "normal" albumin in its ability to bind dyes or react with iodoacetate[6]; the methods used to obtain these data may not have been sufficiently sensitive to detect existing differences.

Future work will show the significance of these findings for cancer diagnosis. The number of samples is still too small to make any conclusions. Moreover, the optical activities and stabilities toward denaturing agents of serum proteins in

(12) E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, THIS JOURNAL, **69**, 1753 (1947).

(13) L. F. Hewitt, *Biochem. J.*, **21**, 216 (1927).

diseases other than cancer are not known. The work will be continued along these lines, and attempts will be made to find differences by means other than optical rotation.

Acknowledgments.—The able technical assistance of Miss Elouise Oliver is acknowledged. We appreciate also the coöperation of Drs. C. D. Howe and B. J. Trunnell of the Anderson Hospital in providing the blood samples.

HOUSTON, TEXAS

NOTES

8-Azaguanine Analogs[1,2]

BY CARL TABB BAHNER, DOROTHY ELLIS BILANCIO AND EMMA MARGARET BROWN

RECEIVED SEPTEMBER 21, 1953

The effects of 8-azaguanine[3] as an inhibitor of the growth of microörganisms and certain tumors led to a request that we prepare similar compounds for studies which might throw light on the relation of structure to biological activity. As one of the simplest possible changes we undertook to replace the oxygen atom by a sulfur atom. Klingsberg and Papa[4] have reported the use of a pyridine solution of P_2S_5 for replacing the oxygen atom in 3,5-diiodo-2-pyridone and other compounds which are soluble in pyridine. 8-Azaguanine is practically insoluble in pyridine, but dissolves in a hot solution of P_2S_5 in pyridine. 5-Amino-7-mercapto-1-v-triazolo(d)pyrimidine and 5,7-dimercapto-1-v-triazolo(d)pyrimidine have been prepared from 8-azaguanine by taking advantage of this fact.

5-Amino-7-mercapto-1-v-triazolo(d)pyrimidine.—Thirteen grams of 8-azaguanine was added rapidly to a solution of 27 g. of P_2S_5 in 300 g. of pyridine. As refluxing was continued the clear, brown solution began to deposit crystals. After 6 hours the hot mixture was poured into 640 ml. of boiling water. Upon cooling and filtering 10 g. of buff colored solid was obtained. The crude solid which consisted partly of a phosphorus-containing compound was treated with boiling water. The crystals which deposited on cooling the water were dissolved in hot 0.05 *N* KSH. The precipitate which appeared upon acidification of the KSH solution with acetic acid and cooling was dried with care to avoid atmospheric oxidation and the methanol soluble fraction was recrystallized to give 2 g. of a final product which decomposed at 270°. In paper chromatography using a solvent consisting of 60 ml. of water, 3.6 ml. of acetic acid and 300 ml. of *n*-butanol, the R_f was 0.57; ultraviolet absorption: at *p*H 10 log $E_{224\ m\mu}$ 4.132, log E_{325} 3.950; at *p*H 6.51 log E_{231} 4.097, log E_{341} 3.925. *Anal.* Calcd. for $C_4H_4N_6S$: C, 28.51; H, 2.39; N, 49.97. Found: C, 28.39; H, 2.45; N, 49.81.

5,7-Dimercapto-1-v-triazolo(d)pyrimidine.—The crude solid obtained by a single treatment of 13.0 g. of 8-azaguanine with P_2S_5 in pyridine was dissolved in hot 1:1 hydrochloric acid and thrown out of solution by neutralization with ammonia. Six and seven-tenths grams of the recrystallized material was added to a solution of 11.0 g. of P_2S_5 in pyridine. After refluxing the mixture for 6 hours it was poured into boiling water and the crystals which formed were recrystallized by dissolving in hot 1:1 HCl and neutralizing with ammonia; yield 1 g. The R_f for this compound, using butanol–acetic acid–water solvent, was 0.76; ultraviolet absorption: at *p*H 6.51 log E_{283} 4.153, log E_{343} 4.002; at *p*H 10.0 log E_{283} 4.076, log E_{343} 3.801. *Anal.* Calcd. for $C_4H_3N_5S_2$: C, 25.95; H, 1.63; S, 34.60. Found: C, 26.20; H, 1.88; S, 34.58.

Data on the biological effects of these compounds are to be reported elsewhere.

We are indebted to Dr. R. O. Roblin and Dr. J. M. Ruegsegger of Lederle Laboratories for the 8-azaguanine used in these experiments, to Oldbury Electro-Chemical Company for phosphorus pentasulfide, to Dr. Alfred Gellhorn of Columbia University Institute of Cancer Research for calling our attention to the need for substituted triazolopyrimidines in his study of the mechanism of action of 8-azaguanine, to Dr. Howard Skipper and Dr. Lee Bennett and their associates of Southern Research Institute for determining the ultraviolet absorption spectra of these compounds and screening them against certain tumors, and to Dr. Harry W. Galbraith of Galbraith Analytical Laboratories for the carbon, hydrogen, nitrogen and sulfur analyses.

DEPARTMENT OF CHEMISTRY
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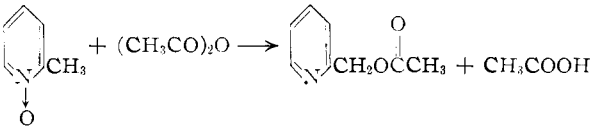
A New Synthesis of 1-(2-Pyridyl)-alkanols

BY O. H. BULLITT, JR., AND J. T. MAYNARD

RECEIVED DECEMBER 12, 1953

During an investigation of some of the reactions of pyridine N-oxides, a new rearrangement of alkyl-substituted pyridine oxides was encountered. The rearrangement is promoted by carboxylic acid anhydrides and results in the formation of an acylated 1-(2-pyridyl)-alkanol. For example, 2-methylpyridine oxide reacts with acetic anhydride to give 2-pyridylmethyl acetate



Proof of the proposed structure was provided by comparison of ultraviolet (Table I) and infrared spectra with those of known compounds, elementary analysis and preparation of the known picrate of the 2-pyridylmethanol obtained by saponification of the acetate.

(1) This research was supported in part by grants from the Damon Runyon Memorial Fund for Cancer Research and the National Institutes of Health, U. S. Public Health Service.

(2) Presented in part at the Southeastern Regional Meeting of the American Chemical Society, Auburn, Alabama, October 24, 1952.

(3) R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughn, Jr., THIS JOURNAL, **67**, 290 (1945).

(4) E. Klingsberg and D. Papa, *ibid.*, **73**, 4988 (1951).