TABLE I

PER CENT. COMPOSITION BASED ON TOTAL 260 mμ ABSORPTION[a]

Component	R_f value, paper chromatogram	H_4ATP Paper	H_4ATP Ion exch.	Ba_2ATP Paper	Ba_2ATP Ion exch.	Na_4ATP #1[c] Paper	Na_4ATP #1[c] Ion exch.	Na_4ATP #2[d] Paper	Na_4ATP #2[d] Ion exch.[e]	BaADP Paper	BaADP Ion exch.
ATP	0.83	20.6	22.2	81.5	76.5	(±)[b]	2.0	75	61	(±)	0.1
ADP	.77	42.6	40.0	18.5	14.9	(±)	7.2	23	11	90	84
AMP	.69	35.5	25.4	(±)	2.3	95	80.5	(±)	2.2	(±)	5.9
Adenosine	.52	0	0	0	0	0	0.9		0	0	0
Adenine	.38	(±)	5.9	0	0	0	0		0	0	0

[a] Recovery of components from paper chromatogram and ion-exchange resin was between 90–97% based on 260 mμ. absorption of starting material. [b] ± This sign designates 5% or less, an amount detectable but difficult to quantitate with certainty (2–5 μg.) [c] Na salt which had stood at room temperature for about six months (see text). [d] Na salt which had been kept at −35°. [e] Twenty-five per cent. of the material absorbing at 260 mμ was not recovered in any of the expected fractions.

mixture based on dry weight of the starting material. In most samples examined, the total adenine compounds thus determined accounted for 90–95% of the dry weight. No purine or pyrimidine derivatives other than those reported in Table I were found in the commercial preparations examined.

Discussion

Although good agreement on the assays reported in Table I was achieved with the two analytical schemes, it should be pointed out that the ion-exchange analysis is inherently a more sensitive and rigorous analytical technique than paper chromatography and has the additional advantage of permitting a wide range in the concentrations of components to be separated. Paper chromatography appears to be most useful when a rapid, semiquantitative technique for the analysis of a large number of samples is desired, as in following the composition of a preparation of ATP during isolation procedures.

It is clear that the ion-exchange separation may be used equally well as a preparative method; however, no attention has been paid to the removal of impurities not detected by ultraviolet light absorption. Hence, the method must at present be used in conjunction with enzymatic assay to establish the presence or absence of inhibitors, etc., of this kind.

The instability of the sodium salt of ATP has been noted by others[13] and makes imperative the analytical control of this compound before use in enzyme systems.

(13) F. Lipman, personal communication.

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Transetherification Reactions. Thiophenols with Ethyl β-Ethoxyacrylates[1]

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The transetherification of ethyl β-ethoxyacrylate with alcohols and mercaptans yields ethyl β-alkoxyacrylates and ethyl β-alkylthioacrylates, respectively.[2] Attempts to apply this reaction to phenol were unsuccessful. However, various thiophenols with ethyl β-ethoxyacrylate in the presence of sodium bisulfate catalyst readily undergo the transetherification reaction to give the corresponding ethyl β-phenylthioacrylates in good yields. The various ethyl β-phenylthioacrylates are listed in Table I.

Saponification of these esters gave the β-phenylthioacrylic acids. All the acids as isolated from the saponification experiments melted over a wide range. Fractional crystallization of two of these acids, β-phenylthioacrylic acid and β-(*p*-tolylthio)-acrylic acid, gave in each case two fractions of narrow melting range, the major portion being the higher melting forms. These individual components as well as the original mixtures had the same neutral equivalent values, indicating that the fractions of different melting points are *cis–trans* isomers.

β-Phenylthioacrylic acid was converted to the acid chloride by treatment with phosphorus pentachloride and the resulting acid chloride cyclized with aluminum chloride to 1-thiochromone.

Experimental

The following experiment is typical of all the transetherifications.

Ethyl β-Phenylthioacrylate.—Distillation of 110 g. (1 mole) of thiophenol and 144 g. (1 mole) of ethyl β-ethoxyacrylate from 1 g. of sodium bisulfate gave 42 g. (91%) of ethanol and 180 g. of the thioacrylate.

β-Phenylthioacrylic Acid.—A two-phase mixture consisting of 50 g. (0.24 mole) of ethyl β-phenylthioacrylate and 15.8 g. of potassium hydroxide dissolved in 100 ml. of water was stirred and refluxed for two hours to give a homogeneous solution. Acidification of the solution with dilute hydrochloric acid yielded a white solid which was collected on the filter and washed with water; yield 39 g. (90%). A portion of the acid was recrystallized from petroleum ether (b. p. 90–100°), m. p. 75–103°. *Anal.* Calcd. for $C_9H_8O_2S$: S, 17.8; neut. equiv., 180. Found: S, 17.6; neut. equiv., 181.

Fractional recrystallization of 80 g. of the above acid, m. p. 75–103° (obtained in other experiments), from petroleum ether–acetone mixtures gave 63 g. of white crystalline material, m. p. 127–128.5°; neutral equiv. 181.5. Evaporation of the mother liquors gave a solid which was

(1) For the previous paper of this series, see Croxall and Fegley, THIS JOURNAL, **72**, 2888 (1950).

(2) Croxall, Van Hook and Luckenbaugh, THIS JOURNAL, **71**, 2736 (1949).

TABLE I

$RSCH{=}CHCO_2C_2H_5$

R	B. p. (uncor.), °C.	Mm.	$n^{20}D$	Yield, %	Formula	Analyses, % Calcd.	Found
C_6H_5	145–150	2.3	1.5808	87	$C_{11}H_{12}O_2S$	S, 15.4	15.8
p-$CH_3C_6H_4$	137–138	0.5	1.5752	86	$C_{12}H_{14}O_2S$	S, 14.4	14.9
p-ClC_6H_4	147	2	1.5920	82	$C_{11}H_{11}O_2SCl$	S, 13.2	13.7
3,4-$Cl_2C_6H_3$	152–154	1	1.6018	80	$C_{11}H_{10}O_2SCl_2$	Cl, 25.5	25.0

fractionally recrystallized from a petroleum ether–ethylene dichloride mixture. There was obtained 6 g. of a tan solid, m. p. 83–86°, neut. equiv., 180.

β-(*p*-Tolylthio)-acrylic Acid.—Saponification of ethyl β-(*p*-tolylthio)-acrylate gave a 92% yield of the acid, m. p. 94–125°. Recrystallization from benzene gave a major fraction with unaltered melting point. Fractional crystallization of this material from acetone gave a fraction, m. p. 136–138; neut. equiv. 197 (calcd. 194). The filtrate from the benzene recrystallization upon evaporation yielded a solid which was collected on the filter and recrystallized from benzene, m. p. 104–107°; neut. equiv. 199. The major portion of the acid was the higher melting form.

β-(*p*-Chlorophenylthio)-acrylic Acid.—Saponification of ethyl β-(*p*-chlorophenylthio)-acrylate gave the acid in 90% yield; m. p. 98–112°. *Anal.* Calcd. for $C_9H_7O_2SCl$: S, 14.9; Cl, 16.6. Found: S, 14.9; Cl, 16.5.

β-(3,4-Dichlorophenylthio)-acrylic Acid.—This acid was obtained in an 87% yield, m. p. 98–128°. *Anal.* Calcd. for $C_9H_6O_2SCl_2$: Cl, 28.4; S, 12.9. Found: Cl, 27.6; S, 12.5.

1-Thiochromone.—This material was prepared from 75 g. (0.41 mole) of β-phenylthioacrylic acid (m. p. 75–105°) according to the method outlined in "Organic Reactions"[3] for 2-phenyltetralone-1. The yield was 39 g. (57%), b. p. 115–138° (2–3 mm. dec.); m. p. 76–78° (from benzene).[4] *Anal.* Calcd. for C_9H_6SO: S, 19.7. Found: S, 19.4.

(3) W. S. Johnson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 145.

(4) F. Arndt, *et al.*, *Ber.*, **58B**, 1612 (1925).

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X-Ray Analysis of Some Antibiotic Substances

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The determination of the chemical constitution and molecular configuration of antibiotic substances might be expected to have important consequences with respect to our knowledge of the mechanisms involved in microbiological antagonisms and perhaps to the possible synthesis of new antibiotics. Of the major antibiotics, a complete structure determination has been carried out only for penicillin,[1] although the chemical constitution of Chloromycetin has been elucidated.[2]

Recently we have carried out preliminary X-ray investigations of some antibiotic substances which appear to have somewhat similar ranges of antibacterial and antirickettsial activity. Although we have so far been unable to obtain any definite structural information, it seems worthwhile to review here those results which we have obtained at the present stage of the work.

Chloromycetin, or chloramphenicol, $C_{11}H_{12}N_2O_5Cl_2$, has been shown[2] to be the compound D-*threo*-2-dichloroacetamido-1-*p*-nitrophenyl-1,3-propanediol. We have examined crystals of both chloramphenicol and the corresponding bromine substituted derivative, bromamphenicol. Both substances may be crystallized from water in the form of small white needles or laths, which tend to form agglomerates. X-Ray examination shows that the unit cells and space groups are

Compound	Crystal system	a, Å.	b, Å.	c, Å.	Space group	Z
Chloramphenicol	Orthorhomic	17.6	7.35	22.3	$C222_1$	8
Bromamphenicol	Orthorhombic	17.9	7.4	22.1	$C222_1$	8

The observed densities, 1.49 g. cm.$^{-3}$ 1.865 g. cm.$^{-3}$, respectively, indicate the presence of eight molecules in the unit cell. Complete intensity measurements have been made for chloramphenicol and the three Patterson–Harker sections have been computed. The interpretation of these data is complicated by the very high temperature factor observed for the crystals and so far it has not been possible to locate even the chlorine atoms. It may be surmised, however, on the basis of the outstandingly strong (020) reflection (F(020) = 0.43) and the strong negative birefringence of the crystals, that most of the atoms lie in a plane parallel to (010) at either $y = 0$, $y = 1/4$. It is possible to construct a model of the chloramphenicol molecule in which most of the atoms do lie close to a single plane. Intensity measurements for bromamphenicol are not yet completed. In spite of the large temperature factor which is again observed, it is hoped that the presence of the relatively heavy bromine atoms may lead to a direct structure analysis.

The chemical constitution of aureomycin is still unknown. Crystals of the hydrochloride have been obtained from water in the form of yellow diamond shaped plates, although other forms have also been observed. The crystals are orthorhombic with space group $P2_12_12_1$ and cell constants $a = 11.22$Å., $b = 12.89$., Å$c = 15.55$Å. The observed density, 1.52 g. cm.$^{-3}$, leads to a molecular weight of 515 ± 5 for the asymmetric unit,

(1) "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949.

(2) M. C. Rebstock, H. M. Crooks, J. Controulis and Q. R. Bartz, THIS JOURNAL, **71**, 2458 (1949); J. Controulis, M. C. Rebstock and H. M. Crooks, *ibid.*, **71**, 2463 (1949).