The constants here recorded for the seed oil of *Aegle Marmelos*, Corr., are very similar to those of other *Rutaceae* seed oils, as the following examples[3] show.

	% oil	Density
Calodendron capense	59.2	0.9219 (15°)
Limonia Warneckii	38.5	.....
Citrus Limonum	Up to 54	.921–0.9236 (15°)
Citrus Aurantium	54–57	.921–0.926 (15°)

	n_D^t	Sap. val.	I val.
Calodendron capense	1.465	192.6	108.7
Limonia Warneckii	1.4578	188.8	75.2
Citrus Limonum	1.4645–1.465	188–198	103–110
Citrus Aurantium	1.4638–1.4649	193–197	97–105

(3) Grün and Halden, "Analyse der Fette und Wachse," Bd. II, 93–95 (1929).

Extracted Kernels.—The extracted kernels were noteworthy in containing a high percentage of nitrogen: Sample 1, 12.52% and Sample 2, 12.14% (Kjeldahl), indicating the presence of over 70% protein, probably a globulin since it is largely soluble in cold molar sodium chloride solution.

It is not proposed to continue work on this material.

Summary

The seed oil of *Aegle Marmelos*, Corr., has been examined and the analytical constants and approximate composition recorded.

LUNUWILA, CEYLON RECEIVED NOVEMBER 21, 1934

[CONTRIBUTION FROM THE ORGANIC CHEMICAL LABORATORIES OF THE UNIVERSITY OF FLORIDA]

Benzenesulfonyl Derivatives of *o*-Aminophenol

BY C. B. POLLARD AND L. H. AMUNDSEN

The preparation of *o*-benzenesulfonylaminophenol and *o*-benzenesulfonylaminophenyl benzenesulfonate, m. p. 134°,[1] has been described by Tingle and Williams.[2] Their paper states that their method did not give consistent results, the same method at times giving the first compound and at other times the second.

In this paper favorable conditions are described for controlling at will the formation of either compound obtained by Williams.

In the course of this work *o*-aminophenylbenzenesulfonate was isolated, the existence and stability of which is of interest since corresponding esters of carboxylic acids are unstable.[3] This product showed no change during a period of six months. Proof of structure follows from: (1) its solubility in dilute hydrochloric acid and its insolubility in dilute sodium hydroxide and (2) the formation of *o*-benzoylaminophenyl benzenesulfonate in its reaction with benzoyl chloride in ether solution.

Experimental

***o*-Benzenesulfonylaminophenol.**—To 107 g. (nearly 1 mole) of *o*-aminophenol in 350 cc. of dry dioxane or toluene was added 87 g. (0.5 mol.) of benzenesulfonyl chloride and the mixture refluxed for half an hour. The *o*-aminophenol hydrochloride was filtered off and washed with hot dioxane. The product was precipitated with 1.5 liters of water. It was purified in a yield of 104 g. (85%) by dissolving in 10% sodium hydroxide and precipitating with dilute hydrochloric acid, followed by crystallization from toluene, m. p. 141°.

***o*-Benzenesulfonylaminophenyl Benzenesulfonate.**—Twenty grams of *o*-aminophenol was mixed with 400 cc. of a 10% sodium carbonate solution and 65 g. (2 molecular proportions) of benzenesulfonyl chloride. The mixture was put into a 300-cc. suction flask having the side arm closed with a piece of rubber tubing and a screw clamp. The air in the flask was swept out with carbon dioxide to avoid the oxidation of the *o*-aminophenol during the experiment and the flask was stoppered tightly and shaken vigorously for thirty minutes. After filtering, the solid product was washed with dilute hydrochloric acid and then with water. The product was purified by crystallization from alcohol. Since the compound is not very soluble it is best to use nearly 20 cc. for every gram of the substance. This gives a very pure product; yield, 68 g. (about 95%); m. p. 134°.

***o*-Aminophenyl Benzenesulfonate.**—Thirty-six grams of *o*-aminophenol was dissolved in 265 cc. of a 10% solution of sodium hydroxide and 58 g. (about one molecular proportion) of benzenesulfonyl chloride was added. The mixture was shaken for thirty minutes. The flask should be of such size that the liquid nearly fills it and the air swept out with carbon dioxide very quickly. The solid material was washed with dilute sodium hydroxide, dissolved in dilute hydrochloric acid, and precipitated with sodium carbonate. Purification was accomplished by repeated crystallizations from a one to one mixture of benzene and commercial hexane. About 10 cc. of the solvent was used per gram. The yield was 66 g. (about 82%).

(1) Incorrectly reported as 81–83° by Georgesco, *Chem. Centr.*, **71**, I, 543 (1900); *Bul. Soc. Scinte*, **8**, 668 (1899).

(2) Tingle and Williams, *Am. Chem. J.*, **37**, 61 (1907).

(3) Ransom, *ibid.*, **23**, 1 (1900).

m. p. 87–87.3°. The compound is soluble in dilute hydrochloric acid, alcohol, ether and benzene; insoluble in dilute sodium hydroxide and petroleum ether.

Anal. Calcd. for $C_{12}H_{11}O_3NS$: N, 5.62; S, 12.87. Found: N, 5.58, 5.60; S, 12.62, 12.67.

o-Benzoylaminophenyl Benzenesulfonate. A. From o-Benzoylaminophenol.—Five grams of *o*-benzoylaminophenol was treated with 5 cc. of 20% sodium hydroxide solution and 4.4 g. of benzenesulfonyl chloride by the Schotten–Baumann reaction. The product was crystallized from hexane; yield, 6.3 g. or 76% of the theoretical; m. p. 90.5–91°. About 200 cc. of hot hexane was required to dissolve each gram of the compound.

B. From o-Aminophenyl Benzenesulfonate.—Ten grams of *o*-aminophenyl benzenesulfonate was refluxed in 150 cc. of ether for two hours with 5.7 g. of benzoyl chloride. The precipitate was filtered off and washed with ether. The ether solution was washed with dilute hydrochloric acid, 5% sodium hydroxide, and water and dried over anhydrous calcium chloride. The ether was evaporated off and the product so obtained crystallized repeatedly from hexane; yield, 11 g. or 78% of the theoretical, m. p. 90.5–91°. It is soluble in ether, alcohol and toluene. It is insoluble in water, dilute acid, dilute alkali and petroleum ether. When mixed with the preparation from A the melting point remained unchanged.

Anal. Calcd. for $C_{12}H_{15}O_4NS$: N, 3.96; S, 9.08. Found: N, 3.79, 3.86; S, 8.70, 8.71.

The authors are indebted to Dr. H. G. Shaw and Mr. G. A. Barber for some of the analytical data.

Summary

1. Satisfactory methods have been developed for preparing *o*-benzenesulfonylaminophenol and *o*-benzenesulfonylaminophenyl benzenesulfonate.

2. A new benzenesulfonyl derivative and a new mixed benzoyl benzenesulfonyl derivative of *o*-aminophenol have been prepared and studied.

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[CONTRIBUTION NO. 294 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

The Purification, Specificity and Inhibition of Liver Esterase

BY ZELMA BAKER AND C. G. KING

The primary purpose of the present investigation has been to devise a rapid and uncomplicated method of purifying liver esterase, and then to study the influence of purification upon competitive inhibition and substrate specificity.

The product obtained by Willstätter and Memmen[1] from a procedure involving adsorption, elution and electrodialysis still contained protein material. Using a similar complicated procedure, Kraut and Rubenbauer[2] reported that an esterase preparation had been obtained which was free from protein or protein-like material (apparently based upon a negative Millon reaction in dilute solution). Their product was estimated to be thirty-five times more active than the original extract, and was characterized by a rapid loss in activity after being separated from proteins in solution. It is difficult to evaluate their findings in relation to the degree of purification accomplished per unit of total solids, or to the nitrogen content of the active solution used.

By comparing the activity of enzyme preparations at different stages of purification on a number of possible ester-type substrates, it was possible to differentiate clearly between the ethyl butyrate hydrolyzing enzyme and several other recognized esterases occurring in animal tissues. The primary functional role (*e. g.*, natural substrates) of liver esterase is still largely a matter of conjecture.

The problem of interpreting competitive and non-competitive inhibition phenomena is facilitated by the use of highly purified enzymes, because the surface activity and specific group reactions of concomitant proteins frequently influence the behavior of the enzyme being studied. Our investigations strongly indicate that the liver esterase is itself a typical albumin.

Experimental

Procedure.—The general technique used in preparing an esterase extract of liver powder and in measuring enzyme activity and inhibition has been described previously.[3] After extraction of the enzyme by 0.025 *N* ammonia and precipitation of non-esterase proteins by acetic acid, the solution was 0.4 saturated with sodium sulfate at 37°. The inactive precipitate was removed by filtration and a second precipitation made (*p*H 6.5) by fully saturating with sodium sulfate at 37°. After filtering, the flocculent residue was dissolved in distilled water and dialyzed in a collodion bag against distilled water for three days at 10°, and again filtered. Total organic solids were determined in 20-cc. samples by evaporating to dryness at a tempera-

(1) Willstätter and Memmen, *Z. physiol. Chem.*, **138**, 216 (1924).
(2) Kraut and Rubenbauer, *ibid.*, **178**, 103 (1928).
(3) Glick and King, *J. Biol. Chem.*, **94**, 497 (1931).