those calculated from critical data.[4] The agreement is good for the simpler gases for which equation (7) is valid. For the gases CO_2, N_2O and SO_2, not well represented by the van der Waals equation, the values of a from the critical constants are not satisfactory at temperatures and pressures far from the critical conditions, and hence cannot be expected to agree well with the values of a calculated from other data. It is interesting that PVT data[5] for carbon dioxide at pressures and temperatures more nearly comparable with those of Clark and Katz yield values of a in the neighborhood of 4 to 6, while similar calculations from sulfur dioxide data[6] give values of a as high as 10–15. The values of a from (7) are probably as reliable as any for gases which show large deviations from van der Waals behavior, and like other values of a for such gases, they are useful only over limited ranges of pressure and temperature.

Values of γ at zero pressure can be obtained from molecular and spectral data, and equation (7) should provide a simple, approximate correction to moderate pressures. But it is evident from the table that if values of a are taken from critical data, equation (7) is accurate only for gases which are well represented by the van der Waals equation.

TABLE I

VALUES OF a IN ATM. L.^{2}/MOLES^2

Gas	a (from eq. 7)	a (critical data)
A	1.57	1.345
H_2	0.234	0.244
N_2	1.63	1.390
CO_2	5.02	3.59
N_2O	6.80	3.78
SO_2	19.2	6.71

(4) Lange, "Handbook of Chemistry," fourth ed., Handbook Publishers, Inc., Sandusky, Ohio, 1941, pp. 1307–1309.

(5) Cooper and Maass, *Can. J. Research*, **4**, 283 (1931).

(6) Cooper and Maass, *ibid.*, **4**, 495 (1931).

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[CONTRIBUTION FROM ALLERGEN INVESTIGATIONS, BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE, AND THE ALLERGY CLINIC OF PROVIDENCE HOSPITAL, WASHINGTON, D. C.]

The Chemistry of Allergens. X. Comparison of Chemical and Immunological Properties of CB-1A Preparations from Domestic Castor Beans and Brazilian Castor Bean Pomace[1,2]

BY JOSEPH R. SPIES, E. J. COULSON AND HENRY STEVENS

The allergenic fraction, CB-1A, was originally isolated from a domestic variety of castor beans.[3] Before application of the isolation procedure, it was necessary to shell, grind and defat the seeds. In investigations involving castor beans it is necessary to curtail handling as much as possible because of the hazard associated with the primary toxalbumin, ricin[4,5] and because of the possibility of acquiring sensitivity to the castor bean allergen by continued exposure to dust.[6] In an effort to simplify the procedure and to lessen the hazards involved in isolating a large quantity of CB-1A, an examination of Brazilian castor bean pomace was undertaken. The pomace was used directly without ether extraction, and a preliminary heat treatment was employed to detoxify the ricin.

A yield of 0.45% CB-1A was obtained from the pomace as compared with 1.8% previously obtained from one lot of shelled, defatted, domestic castor beans.[3,7] Results in Table I show the close similarity in chemical composition of the CB-1A obtained from the two sources.

TABLE I

COMPARISON OF CHEMICAL COMPOSITION OF CB-1A FROM SHELLED, DOMESTIC CASTOR BEANS AND FROM BRAZILIAN CASTOR BEAN POMACE

Determination[a]	Composition in Domestic castor beans[b]	% of CB-1A from Brazilian castor bean pomace
Nitrogen	18.4	18.2
Nitrogen pptd. by 5% trichloroacetic acid at 20 ± 0.1°	30.7[c]	39.4
Sulfur	2.33	2.36
Carbohydrate	3.12	3.10
Arginine	26.6	26.6
Cystine	5.0	4.1
Tyrosine	1.1	1.1
Tryptophan	0.0	0.0

[a] Analyses are expressed on an ash-water-free percentage basis. The authors are indebted to Dorris C. Chambers for the microanalytical determinations. Amino acid determinations are expressed on the basis of per cent. of the total nitrogen in the form of the given amino acid. Tyrosine was determined by Lugg's procedure, *Biochem. J.*, **31**, 1422 (1937); **32**, 775 (1938). Other methods were the same as those used in Paper VIII.[3] [b] Isolation of this sample of CB-1A is described in Paper VIII.[3] [c] It was reported in Paper VIII[3] that 22.5% of the nitrogen of CB-1A from domestic castor beans was precipitated by 5% trichloroacetic acid. This value was in error owing to inadvertent use of a lower concentration of trichloroacetic acid.

The samples of CB-1A from both sources were immunologically equivalent. Both samples were equally potent in producing contractions in excised uterine strips of sensitized guinea pigs by the Schultz–Dale technique, using the multiple in-

(1) Not copyrighted.

(2) For Article IX of this series see Spies, Coulson, Chambers, Bernton and Stevens, THIS JOURNAL, **66**, 748 (1944).

(3) Spies and Coulson, *ibid.*, **65**, 1720 (1943).

(4) Stillmark, "Arbeiten des Pharmakologischen Instituts Zu Dorpat," 1889; *Chem. Zentr.*, [2] **60**, 978 (1889).

(5) Osborne, Mendel and Harris, *Am. J. Physiol.*, **14**, 259 (1905).

(6) Figley and Elrod, *J. Am. Med. Assoc.*, **90**, 79 (1928); Vaughn, *J. Allergy*, **1**, 474 (1930); Coca, Walzer and Thommen, "Asthma and Hay Fever in Theory and Practice," Charles C. Thomas, Baltimore, 1931, pp. 42, 175–176, 405.

(7) The lower yield of CB-1A from the pomace is attributed to natural differences which occur in the content of plant constituents from different sources or species and is not attributed to the slightly modified isolation procedure used.

crement titration method.[8] That both samples of CB-1A possessed the same specificity was demonstrated by complete cross-neutralization of excised uterine strips from appropriately sensitized guinea pigs when tested by the Schultz–Dale method.

When diluted $1:10^6$ (near threshold dilution) both samples of CB-1A gave cutaneous reactions of equal intensity on a castor bean-sensitive subject. A threshold quantity of 0.001 mγ of each sample of CB-1A was required to produce positive passive transfer reactions using serum from a castor bean-sensitive subject.[9]

Experimental[10]

Isolation of CB-1A from Brazilian Castor Bean Pomace.—Brazilian castor bean pomace was obtained from a commercial source in the United States. The sample consisted of broken shells and crushed seeds. The pomace was ground to a coarse powder in a hand grinder in a hood.[11] Experiment showed that the yield of CB-1A obtained directly from the pomace was the same as that obtained after ether extraction.

A preliminary heat treatment of the castor beans, to destroy the ricin toxicity,[12] was carried out as follows: To 3 kg. of ground castor bean pomace was added 6 l. of distilled water. The mixture was heated in an autoclave to 85–92° and maintained at that temperature for one and one-half hours. The suspension was then cooled slightly and an additional 12 l. of water was added. The procedure for isolating CB-1A was then essentially the same as that previously described,[3] except that water extracts from 12 kg. of pomace were combined and worked up together. A further convenient modification was the substitution of pressure filtration through a Seitz sterilizing pad, instead of centrifugation in the Sharples supercentrifuge, to clarify solutions at corresponding points in the procedure.

From a total of 39.9 kg. of pomace, worked up in four lots, 181.3 g. (air dried) of CB-1A was obtained. The four samples of CB-1A were combined, dissolved in 2 l. of water and reprecipitated with five volumes of ethanol at 5°. The recovered CB-1A was dried in a vacuum over calcium chloride. Before analysis the dried CB-1A was ground to pass a 100-mesh sieve and equilibrated with air.

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(8) The dilution of each sample of CB-1A required to produce 2 + contractions in the uterine muscles of sensitized guinea pigs was $1:1.83 \times 10^{11}$.

(9) Details of this method of comparing castor bean allergenic fractions are given in Table III of Paper IX.[3] The serum (W) used for this comparison gave positive passive transfer reactions when diluted $1:10^3$. *Cf.* Coca and Grove, *J. Immunol.*, **10**, 445 (1925). The authors are indebted to Dr. Harry S. Bernton for this serum and to Dorris C. Chambers for the clinical tests.

(10) The authors acknowledge the technical assistance of James H. Shimp in the isolation of CB-1A from the pomace.

(11) Ground pomace is commercially available.

(12) Stillmark[4] and later workers have shown that moist heat destroys the toxicity of ricin. Osborne, Mendel and Harris observed that heating at 60–80° coagulates ricin. Unpublished experiments in this Laboratory have shown that heating water extracts of castor beans destroys their toxic action but an ulcer-producing factor remains. Thus guinea pigs survived subcutaneous injection of 174 M. L. D. of nitrogen of a clarified water extract of castor beans that had been heated for one hour at 77–80°. However, ulcers developed at the site of the injection. Carmicheal (*Soc. Exptl. Biol. Med.*, **24**, 5 (1926)) previously reported that ricin solutions were detoxified by sodium ricinoleate but that ulcers always formed at the site of the injection. In protocols of later work by Carmicheal (*J. Pharmacol.*, **35**, 193 (1929); *ibid.*, **35**, 223 (1929)) it is apparent that ulcers sometimes formed on injection of ricin solutions detoxified by other means. Further work is needed to clarify the nature of this ulceration factor produced by detoxifying ricin solutions or castor bean extracts.

The Sulfonation of Acetophenone

BY E. H. WOODRUFF

It was reported[1] that the alkali fusion of acetophenone disulfonyl chloride gave *m*-hydroxybenzoic acid, confirming the statement of Suter and Weston[2] and that only one sulfonic acid group had entered the ring. Weston and Suter,[3] in a more detailed study, isolated only salicylic acid from the fusion of their acid chloride, showing it to be acetophenone 2,α-disulfonyl chloride.

To clarify this contradiction a further examination of the experimental data (not previously reported) yields the following information:

When added to cooled chlorosulfonic acid and then heated at 110° acetophenone yields an ether-insoluble compound, m. p. 195–196° (from carbon tetrachloride), identical with that previously reported.[3,4] This material on fusion gives salicylic acid. If, however, acetophenone is added to chlorosulfonic acid already heated to 110°, upon pouring onto ice almost no insoluble precipitate is obtained. Upon working up the aqueous solution a disodium disulfonate is obtained which on fusion with alkali gives *m*-hydroxybenzoic acid. Thus the *m*-hydroxybenzoic acid does not result from the fusion of the disulfonyl chloride, as the previous report from this Laboratory would indicate, but from another water-soluble product of the sulfonation. The aqueous solution from which the disulfonyl chloride was obtained has not been worked up in a similar manner. It would appear, however, that acetophenone may sulfonate in either the ortho or meta position when treated with chlorosulfonic acid. A low temperature during the mixing of the reactants favors the formation of the ortho derivative. Whether the meta isomer is formed during the process giving the best yield of the ortho isomer has not been demonstrated but in view of the low yield of the ortho isomer it is not unlikely that such is the case. The behavior of acetophenone toward sulfonation appears to duplicate that of nitration[5] where both the ortho and meta nitroacetophenones are formed.

Experimental

Acetophenone 2-α-Disulfonyl Chloride.—This was prepared by the addition of acetophenone to chlorosulfonic acid kept at room temperature and then heated to 110° for one hour[4]; yield 21.5%, m. p. 195–196° uncor., crystallized from carbon tetrachloride.

Acetophenone 3-α-Disulfonic Acid Disodium Salt.—To 1 kg. (8.60 moles) of chlorosulfonic acid heated to 110° with stirring, 168 g. (1.4 moles) of acetophenone was added over a period of one hour. The temperature rose to 120° during the addition and was kept there an additional hour. After cooling to 10°, the material was added to 4 kg. of cracked ice. One hundred cc. of chloroform was added and the solution filtered with suction. Upon heating the solution to 80° with a current of steam, 1880 g. (5.8 moles)

(1) Woodruff, THIS JOURNAL, **64**, 2859 (1942).

(2) Suter and Weston, *ibid.*, **61**, 233 (1939).

(3) Weston and Suter, *ibid.*, **61**, 389 (1939).

(4) Riesz and Frankfurter, *Monatsh.*, **50**, 68 (1928).

(5) Reese, *Chem. Rev.*, **14**, 90 (1934).