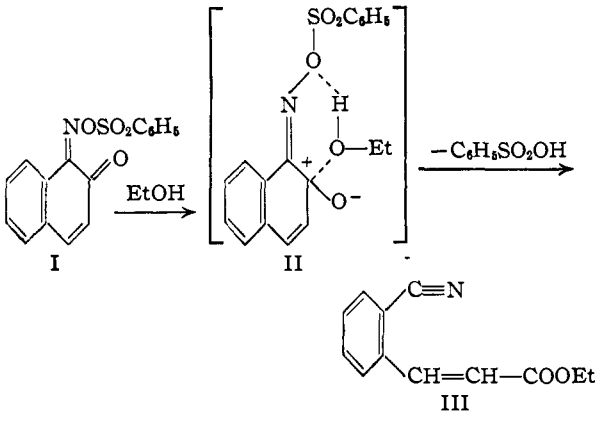


larly, the product, m.p. 45–46°, from the benzenesulfonic ester of 1,2-naphthoquinone-2-oxime, is *o*-carbethoxycinnamonitrile.

The assignment of structure III is consistent with the finding of Werner and Piguet[6] that *trans*-*o*-cyanocinnamic acid was formed by treating 1,2-naphthoquinone-1-oxime (I) with benzenesulfonyl chloride in pyridine solution, followed by acidification of the reaction mixture with dilute sulfuric acid. Also, the product obtained by Beckmann and Liesche,[5] when they subjected 1,2-naphthoquinone-2-oxime to similar reaction conditions, has been identified as *o*-carboxycinnamonitrile.[7] A logical mechanism for the facile ethanolysis of the benzenesulfonic ester of 1,2-naphthoquinone-1-oxime (I) probably involves a cyclic six-membered transition state (II), and a similar transition state can be pictured to account for the formation of *o*-carbethoxycinnamonitrile. One might expect *cis* products to result from these conversions, but isomerization to the *trans* compounds is readily possible in the acidic medium (liberation of benzenesulfonic acid) either at the transition stage or on equilibration of the product. On the basis of the infrared evidence, it is probable that the *trans* forms of both esters were obtained.[8] The spectrum of ethyl *o*-cyanocinnamate showed a band at 967 cm.$^{-1}$, and the same band, though less intense, was found in the spectrum of *o*-carbethoxycinnamonitrile. In neither spectrum was there a band indicative of a *cis* configuration.[9]

Experimental[10]

Benzenesulfonic Ester of 1,2-Naphthoquinone-1-oxime.—A mixture of 12.6 g. (0.073 mole) of 1,2-naphthoquinone-1-oxime[11] and 12.9 g. (0.073 mole) of benzenesulfonyl chloride in 70 ml. of pyridine was allowed to stand until the heat of reaction had subsided. The reaction mixture was diluted to 500 ml. with water, and the precipitate which formed was filtered, washed with water and dried. The product was recrystallized from chloroform–carbon tetrachloride as yellow needles which possessed two melting points. The material melted at 86.5–89°, almost immediately resolidified and melted again at 139.5–141.5° (dec.), (reported[12] for the benzenesulfonic ester of 1-nitroso-2-naphthol, 124–125° and 141°); yield 5.8 g. (26%).

Anal. Calcd. for $C_{16}H_{11}NO_4S$: C, 61.35; H, 3.54; N, 4.37. Found: C, 60.45; H, 3.63; N, 4.31.

The infrared spectrum showed an absorption band at 1648 cm.$^{-1}$, indicating the presence of conjugated C=O.

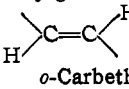
Benzenesulfonic Ester of 1,2-Naphthoquinone-2-oxime.—This compound was prepared from 1,2-naphthoquinone-2-oxime[13] in the same manner as was the benzenesulfonic ester of the 1-oxime. The material was recrystallized as yellow platelets from carbon tetrachloride, m.p. 118–121° (dec.); yield 12.3 g. (54%).

Anal. Calcd. for $C_{16}H_{11}NO_4S$: C, 61.35; H, 3.54; N, 4.37. Found: C, 61.27; H, 3.79; N, 4.56.

Absorption bands at 1685 and 1592 cm.$^{-1}$ in the infrared spectrum indicated the presence of conjugated C=O and conjugated C=N, respectively.

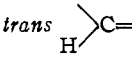
Ethyl *o*-Cyanocinnamate.—To 150 ml. of 95% ethanol was added 5.0 g. (0.016 mole) of the benzenesulfonic ester of 1,2-naphthoquinone-1-oxime. The mixture was boiled until all the material had gone into solution, then for five minutes longer. The solution was decolorized with Norite, filtered and poured into a large excess of cold water. The mixture was cooled to crystallization and the product was separated by filtration. Recrystallization from dilute ethanol gave colorless needles, m.p. 76–77° (reported[8] 57°); yield 2.6 g. (82%).

Anal. Calcd. for $C_{12}H_{11}NO_2$: C, 71.62; H, 5.51; N, 6.96. Found: C, 71.41; H, 5.42; N, 6.91.

The infrared spectrum showed absorption bands at 2220, 1714, 1636, and 967 cm.$^{-1}$, indicative of conjugated C≡N, conjugated ester C=O, conjugated C=C, and *trans*  , respectively.

o-Carbethoxycinnamonitrile.—This ester was obtained from 5.0 g. (0.016 mole) of the benzenesulfonic ester of 1,2-naphthoquinone-2-oxime by the procedure described above for the synthesis of ethyl *o*-cyanocinnamate. Recrystallization from dilute ethanol gave colorless needles, m.p. 45–46°; yield 2.5 g. (79%).

Anal. Calcd. for $C_{12}H_{11}NO_2$: C, 71.62; H, 5.51; N, 6.96. Found: C, 71.78; H, 5.78; N, 7.16.

The infrared spectrum showed the presence of conjugated C≡N (2212 cm.$^{-1}$), and conjugated ester C=O (1712 cm.$^{-1}$). There was also a weak band at 967 cm.$^{-1}$, indicative of *trans*  .

(6) A. Werner and A. Piguet, *Ber.*, **37**, 4295 (1904).

(7) J. W. Curry, Ph.D. Thesis, University of Illinois, 1952.

(8) Ethyl *o*-cyanocinnamate of m.p. 57° has been reported previously (G. Komppa, *Oversikt. Finska Vetenskaps-Soc. Forh.*, **36**, 121). Komppa did not state whether the compound he obtained was the *cis* or the *trans* isomer.

(9) F. A. Miller, in Gilman's "Organic Chemistry. An Advanced Treatise," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 122.

(10) Melting points are corrected.

(11) C. S. Marvel and P. K. Porter, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., Second Edition, 1941, p. 411.

(12) C. A. Edwards, *J. Chem. Soc.*, 813 (1926).

(13) Chao-Lun Tseng and Mei Hu, *J. Chinese Chem. Soc.*, **3**, 60 (1935).

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Ruthenium Isotope Abundances[1]

BY LEWIS FRIEDMAN AND A. P. IRSA

RECEIVED JUNE 17, 1953

Mass spectrographic investigations of the abundances of the ruthenium isotopes have been carried out by Aston[2] and Ewald.[3] Ewald's work, which has been tentatively accepted by the N.R.C. Subcommittee on Nuclear Constants,[4] involved a photographic plate calibration using cadmium abundance data which subsequently have been

(1) Research carried out under the auspices of the U. S. Atomic Energy Commission.

(2) F. W. Aston, *Proc. Roy. Soc.* (*London*), **A132**, 487 (1931).

(3) H. Ewald, *Z. Physik*, **122**, 686 (1944).

(4) K. T. Bainbridge and A. O. C. Nier, Preliminary Report #9, Nuclear Science Series (1950).

revised. Unfortunately insufficient data are available to permit a correction of Ewald's values.

In view of this and the fact that a sizable discrepancy exists between the accepted atomic weight, 101.7,[5] and that calculated from Ewald's data, 101.03, we have reinvestigated the isotope abundances of ruthenium using electrical rather than photographic methods of ion detection.

One of the difficult aspects in determination of the isotopic abundances of ruthenium was the scarcity of volatile compounds from which a stable molecular beam for ionization could be obtained. With the discovery and synthesis of ruthenocene, ($C_{10}H_{10}Ru$),[6] this difficulty is easily circumvented; and isotope abundance measurements can be made easily in a mass range free of background and under circumstances in which errors due to voltage discrimination, fractionation of isotopic molecules, etc., are minimized.

A Nier type mass spectrometer manufactured by the General Electric Company was used. The sample, kindly supplied to us by Prof. Geoffrey Wilkinson of Harvard University, consisted of a few milligrams of solid $C_{10}H_{10}Ru$ which was synthesized from $RuCl_3$ obtained from the American Platinum Works, Newark, N. J. It was placed in a glass tube which could be sealed directly on the head of the mass spectrometer with a waxed ball joint. One end of this tube opened directly to the gas inlet port of the ionization chamber; the other, sample-containing end, was sealed off and projected downward at right angles to the spectrometer tube. The sample was cooled with liquid nitrogen during preliminary evacuation of the spectrometer. An adequate vapor pressure was obtained at room temperature and lower. Runs reported here were made at 15°. The spectrum was taken originally with 50-volt ionizing electrons, 2000-volt ion accelerating potential and magnetic scanning. Isotopic abundances were measured with 10-volt ionizing electrons to eliminate dissociative ionization processes. The technique was calibrated by determining the isotopic composition of mercury with identical sampling and operating procedures. Results on mercury were in agreement with those of Nier[7] within 0.5% except at the relatively rare Hg^{196} and here the values checked to within 1%.

The mass spectrum of ruthenocene obtained with 50-volt electrons from Ru^+ up to and including the molecular ion is presented in Table I. The spectrum reported was partially resolved into a monoisotopic spectrum based on Ru^{104}, C^{12} and H^1. The most interesting features of the spectrum are the relatively large yield of molecular ions, appreciable amounts of doubly charged ions, and the alternation in relative probabilities for the processes involving loss of one, two, three and four carbon fragments. These features are observable in the mass spectra of aromatic fused ring systems, naphthalene and anthracene. Another feature of some practical importance in the determination of isotopic abundances is the extremely small probability of loss of hydrogen from the molecular ions.

(5) E. Wichers, THIS JOURNAL, **74**, 2447 (1952).
(6) G. Wilkinson, *ibid.*, **74**, 6146 (1952).
(7) A. O. C. Nier, *Phys. Rev.*, **79**, 450 (1950).

TABLE I

Mass	Relative intensity	Probable ion formula	Mass	Relative intensity	Probable ion formula
234	100	$C_{10}H_{10}Ru^{104}$	155	1.8	$C_4H_8Ru^{104}$
208	2.6	$C_8H_8Ru^{104}$	143	5.2	$C_3H_8Ru^{104}$
206	5.0	$C_8H_6Ru^{104}$	142	2.8	$C_3H_2Ru^{104}$
193	0.3	$C_7H_5Ru^{104}$	141	14.0	C_3HRu^{104}
180	3.5	$C_6H_6Ru^{104}$	130	1.2	$C_2H_2Ru^{104}$
179	3.5	$C_6H_5Ru^{104}$	117	7.5	$C_{10}H_{10}Ru^{104++}$
169	24.0	$C_5H_5Ru^{104}$	104	6.0	Ru^{104+}

The observed polyisotopic spectrum of the molecular mass from which isotopic abundances were computed is presented in column 1 of Table II. The data were averaged from 14 scans, with average deviations as indicated. Since no detectable ions involving loss of hydrogen from $C_{10}H_{10}Ru^{96}$ were observed with 10-volt electrons the only corrections necessary are those for C^{13} and H^2. The abundance of the latter was assumed to be 0.015% or "natural abundance." A statistical distribution of C^{12} and C^{13} was assumed in computing the natural abundance of C^{13} from the 226 and 227 ($C_{10}^{12}H_{10}^{1}Ru^{96}$ and $C_9^{12}C^{13}H_{10}^{1}Ru^{96}$) peak intensities. The value 1.105% C^{13} was obtained, in excellent agreement with the accepted 1.108%.[4] The experimentally determined C^{13} abundance was used to compute the contribution of molecules containing $2C^{13}$ atoms to the polyisotopic spectrum. Errors arising from the assumption on the relative abundance of H^2, and neglect of more than two C^{13} molecules could have been detected in the internal consistency of peaks computed for ions of mass 231 and 233, $C_9^{12}C^{13}H_{10}Ru^{102+}$ and $C_9^{12}C^{13}H_{10}Ru^{104+}$. The agreement between computed and observed peaks at these masses was better than 1% indicating an approximate 0.1% error in the isotope abundances arising from the C^{13} and H^2 corrections. In turn, 0.1% may be set as an upper limit for the relative abundances of Ru^{97} and Ru^{103}. This particular system is not well suited for the purpose of detecting rare isotopes because of these interferences. The Ru^+ ion yield and doubly charged ruthenocene ions were not useful in estimating isotope abundances because of relatively weak intensity and large backgrounds in these spectral regions.

The abundances of Ru isotopes computed by starting at Ru^{96} and removing the 1.1% C^{13} and 0.015% H^2 from successively higher masses are presented in column 4 of Table II. The work of

TABLE II

Mass	Polyisotopic ruthenocene molecule ions Relative intensity	Mass	Relative abundances ruthenium isotopes This work	Ewald
226	4.923 ± 0.018[a]	96	5.50	5.68
227	0.557 ± .006			
228	1.73 ± .01	98	1.91	2.22
229	11.57 ± .01	99	12.70	12.81
230	12.56 ± .05	100	12.69	12.70
231	16.57 ± .03	101	17.01	16.98
232	30.03 ± .06	102	31.52	31.34
233	3.23 ± .04			
234	16.97 ± .06	104	18.67	18.27
235	1.854 ± .016			

[a] Average deviation.

Ewald is presented in the fifth column. In general the agreement is good, with differences in the direction expected from the revision of Nier's cadmium data. That is, low abundance nuclides are moderately reduced with higher abundance nuclides correspondingly increased. There is a moderately large percentage difference at Ru^{98}. This was a weak line in Ewald's spectrum and most susceptible to error in plate calibration and densitometry. Variations in the natural abundance of Ru^{98} are of interest as a possible means of detection of natural Tc^{98}.

The atomic weights computed from these data and Ewald's are in good agreement. Using the packing fraction listed by Mattauch[8] and the chemical conversion factor of 1.000275 a value of 101.08 is obtained. This agrees with the value of 101.08 obtained by Gleu and Rehm[9] from studies on the decomposition of $RuCl_3 \cdot 5NH_3$ but seriously diverges from the accepted value, 101.7, obtained from studies on oxide decomposition.

The authors wish to thank Prof. Geoffrey Wilkinson for his coöperation in supplying us with a sample of pure ruthenocene.

(8) T. Mattauch and S. Pluegge, "Nuclear Physics Tables," Interscience Publishers, Inc., New York, N. Y., 1942.

(9) K. Gleu and K. Rehm, *Z. anorg. Chem.*, **235**, 352 (1938).

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Competitive Interaction between Proteins and Surface Active Anions Demonstrated by Electrophoresis[1]

BY JOSEPH F. FOSTER AND JEN TSI YANG

RECEIVED MAY 23, 1953

Both ovalbumin[2] and serum albumin[3] possess a strong affinity for anionic detergents which is manifested in the formation of an "all-or-none"[4] complex. This complex formation is thought to involve denaturation of the protein and the denatured complex can be resolved from the native protein electrophoretically. The authors have shown recently[5] that ovalbumin (O) yields the all-or none reaction at lower detergent concentrations than does bovine serum albumin (A).

It occurred to the authors that it should be possible to demonstrate this difference in reactivity directly through electrophoretic studies of mixtures of the proteins. The technique used was to mix one of the native proteins with the dodecylbenzene sodium sulfonate complex of the other. After allowing the mixed solution to stand for two days at 2° it was dialyzed for two days against the electrophoretic buffer (either glycine–NaCl, *p*H 10.0, Γ/2 0.1 or veronal–NaCl, *p*H 8.5, Γ/2 0.1) and subjected to electrophoresis. Some typical results are summarized in Fig. 1.

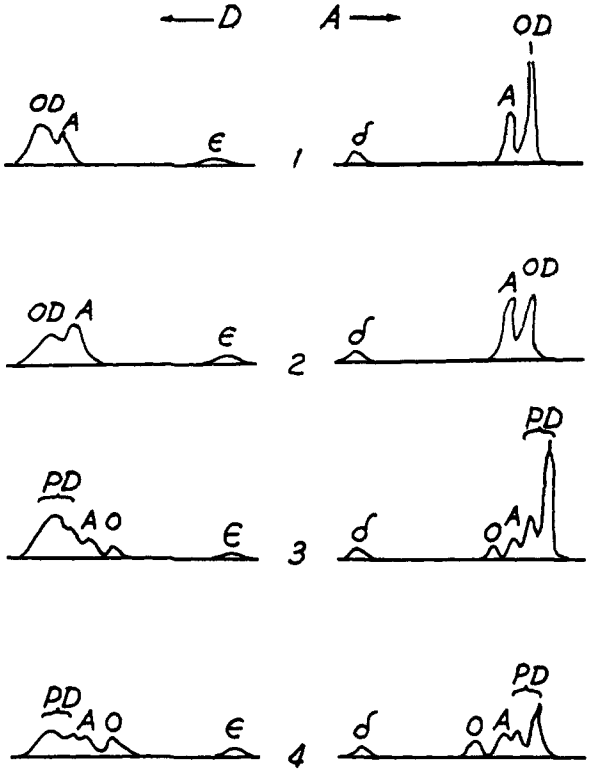

Fig. 1.—Electrophoretic analyses of albumin-SDBS mixtures in glycine–NaCl buffer (*p*H 10.0, Γ/2 0.10). Runs 1 and 2, ovalbumin–SDBS (OD) plus bovine serum albumin (A); O/A ratio 66/34 and 48/52, respectively. Runs 3 and 4, bovine serum albumin–SDBS (AD) plus ovalbumin (O); A/O ratio 66/34 and 49/51, respectively.

It will be seen in Fig. 1 that when A is added to the detergent complex of O (OD) only two components are observed and these have the mobilities characteristic of A and OD. Furthermore the relative areas under the boundaries are close to the relative composition in the original mixture. In other words the pattern is an additive function of the separate patterns on the two components. On the other hand when O is added to AD interaction is clearly revealed. Thus the area corresponding to O is greatly reduced from that which would be expected on the basis of the mixing ratio and a boundary corresponding in mobility to A appears. Similar results were obtained in the veronal buffer; however, in this buffer the patterns were complicated by a split in the protein–detergent boundary and by the appearance of a fast moving boundary anomaly in the descending pattern.

In a study of the electrophoretic analysis of detergent extracts of the corn proteins Foster, Yang and Yui[6] concluded that zein preferentially binds most or all of the detergent present. It was

(1) Journal Paper No. J-2334 of the Iowa Agricultural Experiment Station, Ames, Iowa. Proj. 978. Supported in part by a grant from Swift and Company. Taken from a thesis submitted by Jen Tsi Yang in partial fulfillment of the requirements for the degree Doctor of Philosophy, Iowa State College, 1952.

(2) H. P. Lundgren, D. W. Elam and R. A. O'Connell, *J. Biol. Chem.*, **149**, 183 (1943).

(3) F. W. Putnam and H. Neurath, *ibid.*, **159**, 195 (1945).

(4) The term "all-or-none" as applied to this reaction is in some respects perhaps unfortunate. It is now well known that different complexes are formed in the case of horse serum albumin[3] and that there may be stepwise binding both prior to and following the so-called "all-or-none" step or steps.[5] The term is used in the absence of a better one with these reservations to distinguish binding which leads to new components which are readily resolvable by electrophoresis.

(5) J. T. Yang and J. F. Foster, THIS JOURNAL, **75**, 5560 (1953).

(6) J. F. Foster, J. T. Yang and N. H. Yui, *Cereal Chem.*, **27**, 477 (1950).