[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Polymerization. XIV. The Reactions of Amylsodium with α- and β-Methylstyrenes[1]

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RECEIVED APRIL 3, 1952

When α-methylstyrene is metalated by amylsodium, 29% of the product consists of a replacement of hydrogen by metal and no fraction corresponding to simple addition to the double bond is obtained. The dimer and polymer amounting to nearly half of the product are primary rather than tertiary organosodium compounds judged by esterification of the carboxylated compounds. When β-methylstyrene was similarly metalated, 85% of replacement of hydrogen in the monomer resulted, with very little polymerization. These two methylstyrenes can be looked upon as phenylpropylenes but the corresponding metalated compounds show none of the behavior of allylsodium in an Alfin catalyst. The presence of these salts, particularly the β-isomer, with an Alfin catalyst caused an increase in activity of that agent.

This study originated from the desire to know how the replacement of hydrogen by phenyl in the allyl ion component of an Alfin catalyst affected this reagent, other work[2] having established that the presence of a phenyl group in the alcohol component reduced the activity somewhat and made the composition more critical. The results show that neither metalated hydrocarbon is suitable in an Alfin catalyst, although the presence of some, particularly of the β-isomer, accelerated polymerization of butadiene by the already very fast-acting catalyst by as much as 20 or 30%. In the course of the work the composition of the metalated products was studied by carbonation and the interesting fact was discovered that the α-methylstyrene had undergone nearly 50% dimerization and polymerization when metalated and that the carboxyl group was largely a primary one, judged by partial esterification, rather than tertiary which might have been expected.

The metalations of the methylstyrenes were carried out with amylsodium in the presence of sodium isopropoxide, partly because that salt facilitates the metalating activity of the sodium reagent and partly because of an established practice of preparing all olefin sodium salts in the presence of sodium isopropoxide so that no possible catalytic effect of the Alfin[3] type would be overlooked. The products from α-methylstyrene were carbonated. The unsaturated acids were hydrogenated in order to prevent resinification and were then subjected to a gentle esterification in a solution of dilute hydrogen chloride in methanol—so gentle, indeed, as to esterify only primary acids and not the tertiary ones which would result if addition of a sodium compound had taken place across the double bond, as in equation (1).

$$C_6H_5(CH_3)C{=}CH_2 \xrightarrow{RNa} C_6H_5(CH_3)C(Na){-}CH_2R \xrightarrow{CO_2} C_6H_5(CH_3)C(CO_2Na){-}CH_2R \quad (1)$$

$R = C_5H_{11}$, C_9H_9 or other organic anion

The more resistant acids were subsequently esterified by diazomethane. All esters were fractionally distilled and the fractions examined by saponification, refractive indices, ultimate analyses and, where desirable, by the preparation of suitable derivatives.

The results recorded in Table I show that the dominant metalation was a displacement of hydrogen rather than the addition pictured in equation (1) and that polymerization seemed to have occurred without such an addition. For example, the isolation in fractions 1 and 5 of methyl β-phenyl-*n*-butyrate in 29% yield certifies to a displacement possibly at the terminal methylene group, as in equation (2)

$$C_6H_5(CH_3)C{=}CH_2 \xrightarrow{(1)\ C_5H_{11}Na;\ (2)\ CO_2} C_6H_5(CH_3)C{=}CHCO_2Na \quad (2)$$

because the unsaturated acids which were used in preliminary experiments resinified easily as do α,β-unsaturated acids. However the exact end of the three-carbon system attacked is not a factor in the discussion. The remainder of the easily esterified portion (fractions 2 to 4) also represents primary carboxylates derived by some path other than addition, and fractions 6 and 7 also have analyses which are not compatible with the idea that an amyl radical has added. Thus 71% of the products appears clearly to be derived by paths other than that represented in equation (1).

TABLE I

METHYL ESTERS OF HYDROGENATED ACIDS DERIVED FROM THE METALATION OF α-METHYLSTYRENE

Fract. no.	Products	B.p., °C.	Mm.	Yield G.	%
	Esterified by alc. and acid			51.3	39
1	β-Phenylbutyrate	65	1	25.0	19
2	Dimer monocarboxylate	141–150	1	2.7	2
3	Polymer carboxylate	173–225	1	13.6	10
4	Residue	338		10.0	8
	Esterified by diazomethane			81.6	61
5	β-Phenylbutyrate	46	0.1	14.0	10
6	Intermediate fract.	68–98	.1	14.0	10
7	Monomer dicarboxylate	100–117	.1	16.0	12
8	Dicarboxylates No. 1[a]	117–170	.5	9.0	7
9	Dicarboxylates No. 2[b]	189–257	2.0	20.6	16
10	Residue			8.0	6
	Total products			132.9	100
1, 5	β-Phenyl-*n*-butyrate			39.0	29
1–7	Primary carboxylates			95.4	71
1, 5–7	Monomer products			69.0	51
2–4	Pri. carb. of dimers and polymers			26.3	20
2–4, 8–10	Total dimers and polymers			63.9	49

[a] Distilled through the fractionating column. [b] Distilled from a Claisen flask.

That polymerization also seemed not to require this addition is shown by the presence of fractions 2, 3 and 4 from the easily esterified portion. This

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[1] This work was carried out under the sponsorship of the Office of Synthetic Rubber, Reconstruction Finance Corporation, Washington, D. C.

[2] A. A. Morton, R. P. Welcher, F. W. Collins, S. E. Penner and R. D. Coombs, THIS JOURNAL, **71**, 481 (1949).

[3] A. A. Morton, E. E. Magat and R. L. Letsinger, *ibid.*, **69**, 950 (1947); A. A. Morton, *Ind. Eng. Chem.*, **42**, 1488 (1950).

esterification was less severe than is often employed for the separation of primary from tertiary acids and was not strong enough even to affect all the primary carboxyl groups, as shown by the presence (fraction 5) of nearly a third of the β-phenyl-*n*-butyrate in the less easily esterified portion.

Actually no need exists for assuming that any of the reaction products involved addition of amylsodium to α-methylstyrene because 71% of the products consisted of a displacement of hydrogen as the main reaction and metalations of monoalkylbenzene systems are usually accompanied by a fair amount of further metalation in the aromatic ring itself.[4-6] The aromatic carboxylic acids thus formed would not esterify in this alcohol–acid treatment. All such carboxylates should appear in the diazomethane treated portion and the higher proportion of carbomethoxy groups to carbon in that fraction shows that they did. Further evidence of the aromatic character of these dicarboxylates was furnished by the molecular exaltation to 1.08 for fraction 7, indicative of a carbomethoxy group adjacent to the ring. No fact, therefore, indicates that any part of these reactions proceeds as in equation (1) and many facts testify to the predominance of metalation by replacement of hydrogen.

β-Methylstyrene was attacked easily and the mixture was carbonated. About 85% of the acidic products was a mixture of monomer carboxylic acids of which 32% was separated as 2-phenylbuten-3-oic acid, at least 48% was found as β-benzalpropionic acid and 20% was not characterized. Close to 6% of monomerdicarboxylic acid formed in accord with the vigorous metalating action of the reagent. The polymerization was slight, around 10% of material which seemed to be mostly dimer. Campbell and Young[7], by the action of allylbenzene or β-methylstyrene with sodamide in liquid ammonia obtained a low yield of acids which consisted of about 90% 2-phenylbuten-3-oic acid and 10% benzalpropionic acid. In addition they reported the formation of 30 to 65% of dimer. Thus the heterogeneous system in pentane behaved differently from the homogeneous system in ammonia and favored strongly the metalation by displacement over polymerization.

Metalated Methylstyrenes and Polymerization.—The prospect of using these methylstyrenes for an Alfin catalyst was tested by the two methods used in a previous case,[6] first as organosodium compounds associated with sodium isopropoxide—the condition in which they were obtained by the metalation described above—and second as the compounds made when the hydrocarbons are added to an active Alfin catalyst—a condition which would permit only a partial replacement of the more active of the allyl ion by the phenylallyl or methylstyryl system. Only that allyl ion which is less well incorporated in the Alfin catalyst, as described in the previous section, would probably be reactive.

By the first test no outstanding polymerization of butadiene of the Alfin type was observed. If any was present, the intrinsic viscosity was very low. By the second method both appeared to enhance the polymerization of butadiene, the β-isomer by as much as 20%.

Acknowledgments.—The authors are greatly indebted to Christiana L. Clapp, Jeanne F. Kilduff and Laura W. Blake for assistance in preparation, purification and identification of many compounds, and for measurement of the properties of the polymers. They are also indebted to S. M. Nagy for elemental analyses.

Experiments

Metalation of α-Methylstyrene.—α-Methylstyrene was obtained from Eastman Kodak Company and was fractionated, n^{25}D 1.5353–1.5356, recorded[20] 1.5358. The hydrocarbon (88.5 g. or 0.75 mole) was added dropwise over a 30-minute period at 20 ± 5° to a mixture of amylsodium and sodium isopropoxide prepared[8] from 3 g. atoms of sodium, 1.5 moles of *n*-amyl chloride and 0.60 mole of isopropyl alcohol. The mixture was stirred for 6.5 more hours, allowed to stand overnight and then forced onto solid carbon dioxide. A total of 173 g. of acids from two such metalations was dissolved in 500 ml. of methanol and was catalytically hydrogenated (0.43 mole of hydrogen was absorbed) over 5 g. of 10% palladium-on-barium sulfate. This hydrogenated acid was then partially esterified in two separate lots in one of which 122 g. of the acid in 2 l. of 0.0207 *N* hydrochloric acid–methanol was allowed to stand for 95 min. at 25° and in the other 51 g. in 1 l. of 0.0221 *N* hydrochloric acid–methanol solution remained for 89 min. Following each esterification, the mixture was poured into a sodium carbonate solution and the ester separated from the acid. The yields of ester were 37.5 and 15.5 g., respectively. The 101 g. of unesterified acid was again subjected to esterification (1 l. of 0.0207 *N* hydrochloric acid–methanol) for 98 min. to give 9.8 g. of ester, a far smaller amount than in each of the initial treatments. These fractional esterifications were based on the work of Menschutkin,[9] of Sudborough and co-workers,[10] and of Hinshelwood and co-workers,[11] which showed that primary acids only would be esterified and that secondary aliphatic, tertiary aliphatic and benzoic acid should not be affected. The success of the method was shown by the fact that all of the caproic acid was esterified. Part of the β-phenyl-*n*-butyric acid was unaffected because the β-phenyl group lowers the rate of esterification much the way that β-alkyl substituents[12] do. The remaining acids were converted to the methyl esters by treatment with diazomethane.[13]

The 62.8 g. of ester from the easily esterified portion was fractionated through a four-foot column of the Podbielniak type—but with a glass spiral—and the boiling points and refractive indices were observed for each 1- to 4-g. fraction. Methyl caproate (1.6 g.) was collected at 38–41° (24 mm.) and identified by the *p*-phenylphenacyl ester derivative. The fractions derived from α-methylstyrene were then collected as indicated in Table I. The methyl β-phenyl-*n*-butyrate was identified by its saponification equivalent and the melting point of the anilide (136.8–137.6°, recorded[14] 136–137°). The amide melted from 104.3–106.3° (re-

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(4) A. A. Morton and F. Fallwell, Jr., THIS JOURNAL, **60**, 1924 (1938).

(5) A. A. Morton, E. L. Little, Jr., and W. O. Strong, Jr., *ibid.*, **65**, 1339 (1943).

(6) A. A. Morton and E. L. Little, Jr., *ibid.*, **71**, 487 (1949).

(7) T. W. Campbell and W. G. Young, *ibid.*, **69**, 688, 3066 (1947).

(8) A. A. Morton, *et al.*, *ibid.*, **72**, 3785 (1950).

(9) N. Menschutkin, *Ann.*, **195**, 334 (1879); **197**, 193 (1879); *Ann. chim. phys.*, **23** [V], 14 (1881).

(10) J. J. Sudborough and L. L. Lloyd, *J. Chem. Soc.*, **75**, 467 (1899); J. J. Sudborough and M. K. Tutner, *ibid.*, **101**, 237 (1912).

(11) M. K. Williamson and C. N. Hinshelwood, *Trans. Faraday Soc.*, **30**, 1145 (1934); C. N. Hinshelwood and A. R. Legard, *J. Chem. Soc.*, 1588 (1935).

(12) H. A. Smith, THIS JOURNAL, **62**, 1136 (1940).

(13) F. Arndt, C. R. Noller and I. Bergsteinsson, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 165.

(14) J. F. Eijkman, *Chem. Weekblad*, **5**, 655 (1908); *Chem. Centr.*, **79**, II, 1100 (1908).

corded[15] 105°) but the *p*-bromophenacyl ester, hitherto unreported, melted more sharply (60.1–61.2°) after crystallization from hexane.

Anal. Calcd. for $C_{18}H_{17}O_3Br$: C, 59.86; H, 4.75; Br, 22.13. Found: C, 59.67; H, 4.85; Br, 22.02.

The fraction which appeared to be largely dimer monocarboxylate had saponification equivalents that ranged from 313 to 346 (calculated for $C_{20}H_{24}O_2$, 296). The carbon ranged from 80.8 to 81.6% and the hydrogen from 8.0 to 8.1%. The calculated values are 81.04 and 8.16, respectively. The polymer carboxylate fraction had saponification equivalents that ranged from 700–2100. Partial pyrolysis may have occurred.

The esters, 84.9 g., from the diazomethane treatment were distilled at first from a Claisen flask. The 56.3 g. of lower boiling material (up to 189° (2–3 mm.)) was then refractionated through the Podbielniak column. The monomer dicarboxylate (fraction 7) had saponification equivalents which ranged from 118 to 122 (calcd. for $C_{13}H_{16}O_4$, 118.1). The carbon was 65.80% (calcd. 66.08) and the hydrogen was 6.80% (calcd. 6.83%). This material formed a dianilide in 19% yield which had correct analyses for nitrogen (N, 7.86; calcd. for $C_{23}H_{22}N_2O_2$: N, 7.82) but the melting point ranged from 161.7 to 163.7, possibly because the ring metalation had been a mixture of para and meta. The sixth fraction boiled between the β-phenylbutyrate and the monomerdicarboxylate and was probably a mixture of the two. The carbon and hydrogen analyses and saponification equivalents of fractions 8 and 9 indicated that they could be dicarboxylates of dimers and trimers.

Metalation of β-Methylstyrene.—The β-methylstyrene was prepared from ethylphenylcarbinol[16] by dehydration at 360 ± 5° (100 mm.) in a 45 × 3 cm. tube filled with a catalyst made from aluminum phosphate on pumice. The product, after fractionation, had n^{25}D 1.5474–1.5476 and n^{20}D 1.5501. The literature[17] values are 1.5420–1.5494 at 20°; 1.5491 at 25°.[7]

In two experiments, 174.8 g. (1.482 moles) was metalated. The conditions were essentially the same as for the α-isomer except that one hour was used for addition of the hydrocarbon and the pea-green mixture was carbonated immediately after the three-hour stirring rather than allowed to stand overnight. When the aqueous layer from each run was acidified and stirred with 300 ml. of pentane in the manner regularly used[8] in these studies, an oily crystalline material was recovered. Filtration yielded some β-benzalpropionic acid which was added to more of the same material recovered later in the process. The oily filtrates were combined. In all, 120 g. of acid was obtained.

These acids were separated by several multiple acid-base extractions[18] accompanied by crystallization at low temperatures from equal parts of pentane and ether or from pentane. The total crude crystalline β-benzalpropionic acid obtained was 36.3 g. which, after recrystallization, melted at 86.2–87.3° (recorded[19] 86 to 89°) and had the correct neutralization equivalent. It was further characterized by the melting points of the amide, the anilide, and the iodolactone, by conversion to α-naphthol and by hydrogenation to a known compound. In all cases the properties of these products agreed with those described in the literature. For necessary comparisons with other acids the *p*-bromophenacyl ester was found to melt at 110.8–111.6°. *Anal.* Calcd. for $C_{18}H_{15}O_3Br$: C, 60.18; H, 4.21; Br, 22.25. Found: C, 60.20; H, 4.35; Br, 21.91.

Another crystalline acid, 2-phenylbutene-3-oic acid-1 or phenylvinylacetic acid, $C_6H_5(CO_2H)CHCH{=}CH_2$, was obtained similarly in 5 g. yield. The crystals melted at 32.7 to 33.7° which is considerably higher than the recorded[20] value 23–24°. The neutralization equivalent was 161.5–162.1 (calcd. 162.2), n^{25}D 1.5308. Reduction of this acid in decane over palladium-on-calcium carbonate yielded α-phenyl-*n*-butyric acid (and a little β-methylatropic acid from isomerization) which had correct melting points for the acid (40–42°) and amide (82.8–83.5°, recorded[21] 83–84°) the latter identical with the amide made by Eastman Kodak Company. The unsaturated acid yielded 19% of formaldehyde, isolated as the dimethon, when ozonized, and a 22% yield of the iodolactone (standard conditions[9] but with 50% excess iodine solution), and a 61% yield of β-methylatropic acid when refluxed with 10% aqueous sodium hydroxide. The anilide, however, melted at 101.5–102.5°, which is higher than the recorded[21] value of 97–98°. The difficulty experienced in obtaining melting points which agreed with recorded ones and were constant may be caused by dimorphism or in other cases by *cis–trans* isomerism. The *p*-bromophenacyl ester has generally proven more satisfactory for characterization and was found to melt at 65.3–66.3°, after crystallization from hexane. *Anal.* Calcd. for $C_{18}H_{15}O_3Br$: C, 60.18; H, 4.21; Br, 22.25. Found: C, 60.05; H, 4.29; Br, 22.27.

More of this acid was present but was difficult to isolate because of its low melting point. Accordingly the remaining acids were heated with 25 to 30% potassium hydroxide for 3.5 to 5 hours in order to isomerize them to the high melting point (137.1–138.1°, recorded[22] 135–137°) of β-methylatropic acid of which 9.4 g. was obtained. However, the conversion of this acid to the anilide gave a product which melted unexpectedly low at 100.7 to 102.2°, after crystallization from *t*-butyl alcohol and water. The recorded[21] value is 192°. A second preparation of this anilide failed to change the value. The melting point did not change after the sample had been heated in a sealed evacuated tube for two hours at 205–210°. Mixed melting points with the anilides from the other two acids showed a depression. *Anal.* Calcd. for $C_{16}H_{15}ON$: C, 80.98; H, 6.37; N, 5.90. Found: C, 81.19; H, 6.66; N, 6.23. The amide melted at 99.2–100.2° (cor.) (recorded[23] 98–99° and 103–104°). The β-bromophenacyl ester derivative was obtained in two crystalline forms, a needle form (m.p. 78°) from hexane which changed to a rectangular tablet form (m.p. 86.6–87.8°) upon melting or standing in the solvent at room temperature. *Anal.* Calcd. for $C_{18}H_{15}O_3Br$: C, 60.18; H, 4.21; Br, 22.25. Found: C, 59.83; H, 4.27; Br, 22.23. The identity of the acid itself was further confirmed by hydrogenation over 10% palladium-on-barium sulfate (a 1% palladium catalyst proved ineffective) to the expected saturated acid, by ozonization to give a 40% yield of acetaldehyde (isolated as the dimethon which melted at 176.8–177.6° (cor.)) and by failure to give an iodolactone after 32 hours under conditions here used. The difficulty with obtaining values which agreed with those published and the tendency to obtain different crystalline forms in many derivatives of the three acids prompted measurements of the ultraviolet absorption in 95% alcohol (see Table II).

TABLE II

ULTRAVIOLET ABSORPTION MAXIMA OF THREE ISOMERIC PHENYLBUTENOIC ACIDS AND ONE ESTER

	Wave lengths, mμ	ε, mol
$C_6H_5CH{=}CHCH_3CO_2H$	292	970
	283	1530
	250	17500
$C_6H_5(CO_2H)CHCH{=}CH_2$	265	188
	259	246
	253	213
$C_6H_5(CO_2H)C{=}CHCH_3$	None	
$C_6H_5CH{=}CHCH_2CO_2CH_3$	292	1440
	283	1920
	250	16900

This method of separation of pure acids was supplemented by converting all of the acids from a separate preparation to the methyl esters by way of the silver salt and

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(15) E. P. Kohler and M. Reimer, *Am. Chem. J.*, **33**, 353 (1905).

(16) V. Grignard, *Chem. Centr.*, **II**, 623 (1901).

(17) G. Egloff, "Physical Constants of Hydrocarbons," Reinhold Publishing Corp., New York, N. Y., 1946–1947.

(18) A. A. Morton, "Laboratory Technique in Organic Chemistry," McGraw–Hill Book Co., Inc., New York, N. Y., 1938.

(19) R. Fittig and H. W. Jayne, *Ann.*, **216**, 98 (1882); R. P. Linstead and L. T. D. Williams, *J. Chem. Soc.*, 2741 (1926); J. C. Ambeland and T. B. Johnson, THIS JOURNAL, **61**, 74 (1939).

(20) H. Gilman and S. A. Harris, *ibid.*, **53**, 3541 (1931).

(21) C. R. Hauser, P. S. Shell, R. D. Bright and W. B. Renfrew, *ibid.*, **69**, 591 (1947).

(22) A. Oglialoro, *Gazz. chim. ital.*, **15**, 514 (1885); H. Rupe and E. Busolt, *Ann.*, **369**, 332 (1909); E. C. Knowles and J. B. Cloke, THIS JOURNAL, **54**, 2035 (1932); M. A. Phillips, *J. Chem. Soc.*, 220 (1942).

(23) P. Pfeiffer, I. Engelhard and W. Alfues, *Ann.*, **467**, 189 (1928); M. A. Phillips, *J. Chem. Soc.*, 220 (1942).

methyl iodide. A portion (84.3 g.) of the esters when fractionated yielded 3% methyl caproate, 26° (4 mm.), 26% methyl 2-phenylbuten-3-oiate, 84°(2.7 mm.), 17% of unidentified monomethyl esters, 88° (1.5 mm.), 39% methyl β-benzalpropionate, 103° (1.9 mm.), 4% of an unidentified fraction 110° (1.8 mm.), a 5% methyl ester of a dicarboxylic acid of composition approximately $C_{13}H_{14}O_4$, *ca.* 125° (0.5 mm.), 5% (determined by calculation of saponification equivalents) and 6% of residue. The unidentified monomethyl esters in fraction 3 gave some evidence that suggested *o*-propenylbenzoic acid might be present but the isolation of pure material proved more difficult than the time available for the work permitted.

Activity of the Methylstyrenes in Polymerization of Butadiene.—α-Methylstyrene, 0.25 mole, was metalated by amylsodium prepared from 1 g. atom of sodium, 0.52 mole of amyl chloride and 0.20 mole of isopropyl alcohol in 500 ml. of pentane. This mixture was allowed to stand 9 days before its activity in polymerization of butadiene by bottle polymerization was tested. The conversions were 10, 30, 80 and 100% in about 60 minutes when 10, 20, 40 and 60 ml. of the reagent were used. The corresponding intrinsic viscosities of these four polymers were 0.40, 0.34, 0.38 and 0.39. The corresponding gels were 17, 0, 19 and 17%.

Three similar preparations of β-methylstyrenylsodium reagents were made but the third one gave typical results. The conversions of butadiene to a polymer were 14, 34, 80 and 90% as 10, 20, 40 and 60 ml. of the reagent were used. The corresponding intrinsic viscosities were 1.5, 1.7, 0.9 and 1.0 and the corresponding gels were 26, 32, 28 and 43%.

The effect of the methylstyrenes was tested also on an Alfin catalyst of known activity prepared in the usual way (two separate preparations) from a total of 6 g. atoms of sodium, 3 moles of amyl chloride and 1.2 moles of isopropyl alcohol, and gaseous propylene. The whole was diluted to a volume of 5250 ml. and stored in a well stoppered bottle. This master batch, which should have a concentration of 0.23 milliequivalent of allylsodium per ml. was treated in 250-ml. aliquots with 0, 1.69, 3.38, 6.67 and 13.5 g. of α-methylstyrene, the last quantity being twice the amount needed to react with all of the allylsodium. Each of these five aliquot portions was stirred for one hour at 8000–9000 r.p.m. at 25 ± 5° and then transferred to well stoppered bottles and diluted to 350 ml. with dried *n*-pentane. Tests for the catalytic activity caused by 5 ml. of the reagent on 30 ml. of butadiene in 210 ml. of pentane were made at the end of 7, 29 and 167 days. The control (or no. 1) caused 64, 39 and 41% of polymerization. The corresponding intrinsic viscosities of the polymers were 14, 11 and 11 and the gel contents were 84, 22 and 60. The fifth reagent, that is, the one with the most α-methylstyrene caused a little more polymerization, 66, 61 and 57%. The corresponding intrinsic viscosities were 16, 13 and 12 and the gels were 50, 43 and 47. The intermediate preparations gave conversions and other values within this general range. Actually, out of a total of twelve tests, the controls were exceeded seven times.

The effect of β-methylstyrene was tested in the same way but the catalysts were allowed to stand for 1 and 36 days before each test. The control (or No. 1) showed 45 and 35% conversions. The intrinsic viscosities were 19 in both cases and the gels were 77 and 80%. The fifth sample, which contained the largest amount of β-methylstyrene, caused 57 and 39% polymerizations. The corresponding intrinsic viscosities were 18 and 16 and the gels were 50 and 42%. In every test (eight comparisons) the reagents with β-methylstyrene showed an increase over the corresponding control, and the usual increase was 20 to 30%.

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[CONTRIBUTION FROM THE INSTITUTE FOR ENZYME RESEARCH, UNIVERSITY OF WISCONSIN]

The Isolation of Flavin Nucleotides[1]

BY E. DIMANT,[2] D. R. SANADI[3] AND F. M. HUENNEKENS[4]

RECEIVED MARCH 17, 1952

Based upon chromatographic methods a procedure is described for the isolation of the coenzyme, flavin-adenine-dinucleotide (FAD), from animal tissues and yeast. The authenticity and purity of the product are established by means of (a) enzymatic assay; (b) absorption spectrum; and (c) paper chromatography in several solvent systems.

The yellow compound, flavin-adenine-dinucleotide (FAD),[5] which functions as the prosthetic group for many oxidative enzymes, was first isolated and characterized by Warburg and Christian.[6,7] Their method consists of extracting the flavins from yeast or animal tissues with hot water and trichloroacetic acid and subsequently purifying the FAD by means of repeated extractions into phenolic solvents, selective precipitation as the silver, lead or mercury complexes, and, finally, recrystallization of the barium complex.

The ultimate preparation of pure FAD by the above method is partially offset by the disadvantages of a low over-all yield and by the contamination with other adenine nucleotides until the final stages of purification.

In an effort to overcome these difficulties a method has been devised which makes use, principally, of the following chromatographic techniques: (1) adsorption chromatography on Florisil which separates the extracted flavins from other nucleotides; and (2) partition chromatography between phenol–butanol and water on Celite, or adsorption chromatography on dicalcium phosphate, which separates the various flavins.

The FAD is obtained in a 40–50% over-all yield and has a dry weight purity of 0.40–0.60; the remaining impurity is innocuous in various enzymatic test systems. The quantitative data on yield, purity, *R* value, *etc.*, given for the various procedures in the experimental section, are, unless otherwise specified, average values derived from many individual preparations.

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[1] A report of this work was presented earlier—D. R. Sanadi and F. M. Huennekens, 117th Meeting of the American Chemical Society, Detroit, Mich., April, 1950, Abstracts, p. 60C.

[2] Fellow of the National Heart Foundation, U. S. Public Health Service, Federal Security Agency.

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[5] The following abbreviations will be used throughout this paper: FAD, flavin-adenine-dinucleotide; FMN, flavin mononucleotide; Rb, riboflavin; CoA, coenzyme A; AMP, adenosine-5-phosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

[6] O. Warburg and W. Christian, *Biochem. Z.*, **298**, 150 (1938).

[7] For an excellent review of the flavins and the flavin nucleotides, see H. Theorell, "Die Alloxazin-Proteide (gelbe Fermente)," in Methoden der Fermentforschung, Vol. III, edited by E. Bamann and K. Myrbäck, Photo Offset Reproduction by Academic Press, Inc., New York, N. Y., 1945, pp. 2361–2384.