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12. Kōno Kinoshita and Shoichi Nakajima : Studies on the Structure of Itaconitin. II.[1] Identification of Organic Acids as *p*-Aminoazobenzene Derivatives.

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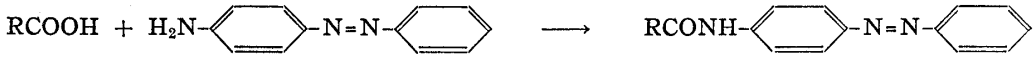
In previous papers of this series, it was reported that itaconitin yielded upon hydrogenation hexahydro derivative as a colorless liquid. Since the hexahydroitaconitin is a lactone with side chains, its oxidation was considered to be of high significance for elucidation of the structure of itaconitin. The oxidation reaction was therefore carried out with potassium permanganate and gave rise to a mixture of colorless organic acids, whose separation was felt rather difficult. With a view to separating this mixture into the components, a new method was devised which would be available for the identification of fatty acids in general.

Many trials have been made for preparing convenient and satisfactory reagents for the identification of organic acids, and it was found, in course of time, that ammonia, aniline, *p*-toluidine, *p*-nitrobenzyl bromide,[2] phenacyl bromide,[3] *p*-halophenacyl bromide,[4] and *p*-phenylphenacyl bromide[5] were the reagents suitable for this purpose as they produced derivatives that were very easy to obtain and crystallized well. However, these derivatives have no color and inconveniences had been felt for separating the mixture of derivatives by chromatographical method. Meanwhile, Masuyama[6] and Sugiyama[7] independently prepared *p*-phenylazophenacyl esters from organic acids by the action of *p*-phenylazophenacyl bromide. The derivatives were applied to chromatographical separation,[6] but the small crystal forms and the low melting points, mostly below 100°, of *p*-phenylazophenacyl esters seemed disadvantageous for the identification of mixed acids.

In this paper will be described some *p*-acylaminoazobenzenes which were formed readily by the reaction of normal fatty acids of $C_1 \sim C_{10}$ with *p*-aminoazobenzene. All the derivatives were obtained in a very good yield and had large crystal form. The



$$R = C_nH_{2n+1} \quad (n=0 \sim 9)$$

melting points of the new derivatives were conspicuously higher than those of the other derivatives, as shown in Table I.

With one exception of formic acid derivative, the figures of the melting points of the new derivatives ascended gradually till 180° of *p*-butyrylaminoazobenzene, then descended, following a characteristic zigzag course till 129° of *p*-caprylaminoazobenzene. The difference in formic acid derivative from other derivatives was also seen in the solubility in chloroform; the former was sparingly soluble while the latter dissolved readily. Variation in the shade of orange color of those derivatives seemed to have an analogous

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1) Part I : This Bulletin, **6**, 31(1958).
2) E. E. Reid : J. Am. Chem. Soc., **39**, 124(1917).
3) B. Rather, E. E. Reid : *Ibid.*, **43**, 629(1921).
4) W. L. Judifind, E. E. Reid : *Ibid.*, **42**, 1043(1920).
5) E. E. Reid : *Ibid.*, **41**, 77(1919).
6) S. Masuyama : Nippon Kagaku Zasshi, **71**, 402(1950).
7) N, Sugiyama, R. Harada, T. Mita, T. Ueno : *Ibid.*, **72**, 152(1951).

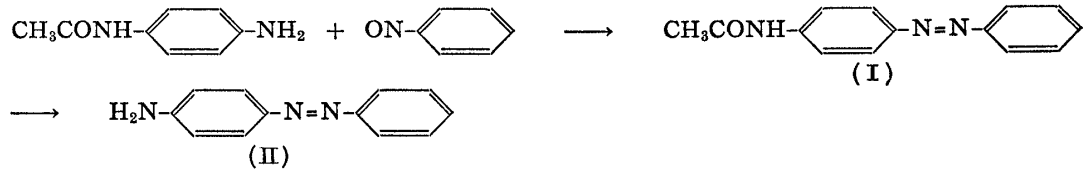
Table I. Melting Point of *p*-Acylaminoazobenzene Derivatives in Comparison with Ordinary Derivatives

Acid \ Deriv.*	I	II	III	IV	V	VI	VII	VIII	IX
Formic	−1	47	53	31	oil	135	74	134	164
Acetic	82	114	147	78	40	86	111	118	141
Propionic	79	103	123	31		63	102	99	169
Butyric	115	95	74	35	oil	63	97	93	180
Valeric	116	63	72			75	64	69	166
Caproic	100	95	75			72	65	64	142
Enanthic	95	71	80		oil	72	62	57	132
Caprylic	106	57	67	oil		67	67	74	136
Pelargonic	99	57	84	〃	oil	69			125
Caprinic	108	63	80	〃		67		66	129

* Derivatives: I, amide; II, anilide; III, *p*-toluide; IV, *p*-nitrobenzyl ester; V, phenacyl ester; VI, *p*-bromophenacyl ester; VII, *p*-phenylphenacyl ester; VIII, *p*-phenylazophenacyl ester; IX, *p*-acylaminoazobenzene.

tendency as the melting point variation; the deepest at butyric acid derivative, turning lighter in propionic and acetic acid derivative, and following the same zigzag course to caprinic acid derivative. Formamidoazobenzene was also an exception in color shade change. This will be a question to be referred to physical method in future.

p-Aminoazobenzene, the starting material for the production of above-mentioned various derivatives, can be obtained by the rearrangement reaction of diazoaminoazobenzene in aniline.[8] *p*-Acetamidoazobenzene (I) was prepared by the condensation of *p*-aminoacetanilide and nitrosobenzene in acetic acid, and hydrolylsis of its product to *p*-aminoazobenzene (II). The yield was almost quantitative in these reactions.



The *p*-aminoazobenzene derivatives of $C_1 \sim C_{10}$ normal fatty acids thus obtained did not change substantially when passed through alumina column and looked promising for application to chromatography. The mixture of *p*-formamido-, *p*-acetamido-, and *p*-propionylamino-azobenzenes was separated clearly into the components by chromatography on alumina.

Because of some difficulty in removing *p*-aminoazobenzene from the reaction mixture, excess fatty acids was used in preparing *p*-acylaminoazobenzenes, but it is clear that the use of equimolar or even less amount of acids is desirable for the purpose of identification of fatty acids. Improvement of this procedure is now on further study.

Experimental*2

Condensation Reaction between *p*-Aminoacetanilide and Nitrosobenzene—The condensation occurred under heat evolution when the suspended mixture of *p*-aminoacetanilide (1.5 g.) and nitrosobenzene (1.0 g.) in AcOH (2 cc.) was warmed on water bath for 5 min. After cool, the reaction mixture was poured into 20 cc. of water and neutralized with 10% Na_2CO_3 solution. The precipitate that deposited was collected by filtration, washed with water, and dried to yield 2.1 g. of crude *p*-acetamidoazobenzene (I), which recrystallized from MeOH to orange-yellow needles, m.p. 141°. *Anal.* Calcd. for $C_{14}H_{13}ON_3$: C, 70.27; H, 5.48; N, 17.56. Found: C, 69.82; H, 5.13; N, 17.95.

Hydrolysis of *p*-Acetamidoazobenzene (I)—(I) (1.3 g.) was suspended in a mixture of conc. HCl (13 cc.) and water (6 cc.), and refluxed for 10 min. under good stirring, when the whole reaction mix-

*2 All melting points are uncorrected.

8) N. Staedel, H. Bauer: Ber., **19**, 1953(1886).

ture turned into a red crystalline mud. After cool, the the reaction mixture was adjusted to weak acidity with 10% NH_4OH and the green-yellow crystalline powder (1.1 g.) that separated was dissolved in hot MeOH, treated with carbon, and cooled to give *p*-aminoazobenzene (II) as orange prisms, m.p. 126.5°, undepressed on admixture with *p*-aminoazobenzene obtained by rearrangement reaction of aminoazobenzene.

Preparation of *p*-Aminoazobenzene Derivatives of Fatty Acids—*p*-Aminoazobenzene was heated with excess of fatty acid at the temperature and time specified in Table II. After cool, the reaction mixture was diluted with water, neutralized with 10% Na_2CO_3 solution (Method A), or poured into petr. benzine (Method B), the brown precipitate thereupon separated was collected on filter, washed with water, and dried. The crude *p*-aminoazobenzene derivatives thus obtained were purified by chromatography in $CHCl_3$ or by treatment with activated carbon in some cases. The yield was very good in every cases.

Table II.

Aminoazobenzene deriv.	Reaction Time (hrs.)	Reaction Temp. (°C)	Appearance (Solvent of recrystn.)	Method
p-Formyl	15 (mins.)	reflux	orange plates ($CHCl_3$)	A
p-Acetyl	1	〃	orange-yellow needles (Hydr. MeOH)	〃
p-Propionyl	2	〃	orange plates (Hydr. MeOH)	〃
p-Butyryl	2	〃	orange rhombic crystals (EtOH)	〃
p-Valeryl	2	〃	orange needles (benzene-petr. benzine)	B
p-Caproyl	2	〃	orange plates (MeOH)	A
p-Enanthoyl	2	〃	〃	〃
p-Capryloyl	2	180°	orange plates (benzene)	〃
p-Pelargonoyl	2	180°	pale orange plates (MeOH)	〃
p-Capryl	2	180°	orange needles (MeOH)	〃

Aminozobenzene deriv.		Analysis (%) Calcd.			Found		
		C	H	N	C	H	N
p-Formyl	$C_{13}H_{11}ON_3$	69.32	4.92	18.66	68.99	4.95	18.93
p-Acetyl		(identified through mixed fusion.)					
p-Propionyl	$C_{15}H_{15}ON_3$	71.12	5.97	16.59	71.36	6.16	16.16
p-Butyryl	$C_{16}H_{17}ON_3$	71.88	6.41	15.72	71.83	6.16	15.99
p-Valeryl	$C_{17}H_{19}ON_3$	72.57	6.81	14.94	72.42	6.44	14.77
p-Caproyl	$C_{18}H_{21}ON_3$	73.19	7.17	14.23	73.21	6.81	14.04
p-Enanthoyl	$C_{19}H_{23}ON_3$	73.75	7.49	13.58	73.73	7.09	13.80
p-Capryloyl	$C_{20}H_{25}ON_3$	74.27	7.79	12.99	73.88	7.36	12.73
p-Pelargonoyl	$C_{21}H_{27}ON_3$	74.74	8.07	12.45	74.90	7.81	12.50
p-Capryl	$C_{22}H_{29}ON_3$	75.17	8.32	11.96	74.91	8.21	11.87

Chromatographic Separation of *p*-Aminoazobenzene Derivatives of Formic, Acetic, and Propionic Acids—A mixture of 200 mg. each of *p*-formamidoazobenzene, *p*-acetamidoazobenzene, and *p*-propionylaminoazobenzene was dissolved in 30 cc. of $CHCl_3$, passed through a column (2×25 cm.) of activated alumina, and the column was developed with the same solvent. When the column was eluted with 80 cc. of $CHCl_3$, the mixture was separated into an orange zone of *p*-formamidoazobenzene at 0.5~3 cm. from the upper surface, 3 cm. blank distance, and another orange zone 6~12 cm. from the upper end, which gradually divided into two bands under continuous developement. Following 200 cc. of blank eluate, *p*-propionylaminoazobenzene was eluted out and after that, *p*-acetamidoazobenzene was obtained alone in the effluent.

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Summary

A new method was devised for identification of fatty acids of C_1~C_{10} series as colored *p*-aminoazobenzene derivatives, which were prepared by condensation between *p*-aminoazobenzene and the fatty acid. On account of their high melting point and large crystal form, they were considered to be convenient derivatives for the identification of fatty acids. The mixture of *p*-formamidoazobenzene, *p*-acetamidoazobenzene, and *p*-propionylaminoazobenzene was separated clearly into the components by chromatography on alumina.

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