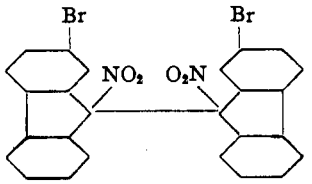
NOTES

2-Bromo-9-Nitrofluorene and 1,1-Dinitro-3,3'-dibromo-bifluorenyl

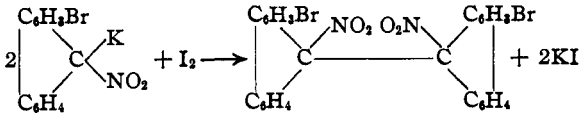
BY C. DALE[1] AND R. L. SHRINER

Recently a compound melting at 170° was described as being 2-bromo-9-nitrofluorene.[2] It is very unusual for a normal nitro compound to melt higher than its aci-form (m. p. 132°). Moreover, the compound decomposes on heating and liberates oxides of nitrogen and forms 2-bromofluorenone. The alkali insolubility of the compound and failure to form a salt of the aci-form, even with potassium methoxide, indicate that it is probably the bimolecular oxidation product with the structure (I) analogous to the product obtained by Nenitzescu[3] in his study of aci-9-nitrofluorene.



I

A reëxamination of this compound has shown that it may be obtained in small amounts by boiling an alcoholic solution of the aci-form, or in good yields by treating the potassium salt of 2-bromo-9-nitrofluorene with one mole of iodine. The product of this reaction is a yellow powder



which shrinks and decomposes over a considerable range of temperature (130–140°). By careful fractionation from methyl acetate, colorless crystals of the compound were obtained. They melted at 172.5° (175° corr.).

The analyses for bromine and nitrogen previously given[2] are correct, but, of course, would check very closely the theoretical values for either the monomolecular or bimolecular structure. A combustion gave the following results: Calcd. for $C_{13}H_8O_2NBr$: C, 53.81; H, 2.76. Calcd. for $C_{26}H_{14}O_4N_2Br_2$: C, 54.00; H, 2.42. Found: C, 54.04; H, 2.51.

The molecular weight was determined by the micro boiling point method, using acetone and benzene as solvents. In acetone a molecular weight of 593 was obtained, and in benzene a value of 554 resulted. The calculated value for I, $C_{26}H_{14}O_4N_2Br_2$, is 578, whereas the molecular weight of $C_{13}H_8BrNO_2$ is 290. It is evident that the compound is the bimolecular oxidation product with the structure I.

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RECEIVED MAY 14, 1936

(1) University of Rochester, Rochester, New York.
(2) Thurston and Shriner, THIS JOURNAL, **57**, 2163 (1935).
(3) Nenitzescu, *Ber.*, **62**, 2669 (1929); **63**, 2484 (1930).

p-Phenylphenacyl Esters of Organic Acids

BY T. LEONARD KELLY AND EUGENE A. MORISANI

During the course of a research the following *p*-phenylphenacyl esters were prepared which do not appear in the literature. Since the melting points of these may be an aid in the identification of acids they are of value.

They were prepared according to the method of Drake and co-workers[1] and were recrystallized to constant melting point. All melting points are

p-PHENYLPHENACYL ESTERS

Acid	M. p., °C.		Halogen, % Calcd.	Found
m-Bromobenzoic	155[a]		20.25	20.13
p-Bromobenzoic	160		20.25	20.16
o-Chlorobenzoic	123		10.12	10.09
m-Chlorobenzoic	154[b]		10.12	10.18
p-Chlorobenzoic	160		10.12	10.19
o-Iodobenzoic	143		28.74	28.38
m-Iodobenzoic	147		28.74	29.21
p-Iodobenzoic	171 (Closed tube)		28.74	28.20
			Nitrogen, %	
o-Nitrobenzoic	140		3.87	3.70
m-Nitrobenzoic	153		3.87	3.95
o-Nitrocinnamic	146		3.61	3.72
m-Nitrocinnamic	193[c]		3.61	3.66
p-Nitrocinnamic	192		3.61	3.94
p-Cyanobenzoic	165		4.10	4.26
Diphenylacetic	111	C.	82.75	82.98
		H.	5.41	5.66

[a] Mixed m. p. with *m*-bromobenzoic acid 127–128°.
[b] Mixed m. p. with *m*-chlorobenzoic acid 130–132°.
[c] Mixed m. p. with *m*-nitrocinnamic acid 180–183°.

(1) Drake and co-workers, THIS JOURNAL, **52**, 3715 (1930); *ibid.*, **54**, 2059 (1932).

uncorrected but were taken in a Fisher melting point apparatus with a set of Anschütz thermometers which gave correct melting points with various pure reagents.

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RECEIVED JUNE 1, 1936

COMMUNICATIONS TO THE EDITOR

SYNTHETIC SUBSTRATES FOR PROTEIN-DIGESTING ENZYMES

Sir:

Knowledge regarding the specificity of those enzymes which hydrolyze intact proteins (peptic, tryptic and catheptic proteinases) is meager. In general it is assumed that these enzymes react exclusively on high molecular substrates.

Recently it has been possible to study the specificity of proteinases with the aid of synthetic substrates. Such substrates have been found in this Laboratory for the catheptic enzymes, papain, liver-cathepsin and bromelin. The authors have now observed the splitting of α-hippuryl-lysine-amide by tryptic proteinase.

α-Hippuryl-ε-carbobenzoxy-lysine methyl ester was converted into α-hippuryl-ε-carbobenzoxy-lysine amide, m. p. 212°, with the aid of methanolic ammonia. *Anal.* Calcd. for $C_{23}H_{28}N_4O_5$: C, 62.7; H, 6.4; N, 12.7. Found: C, 62.6; H, 6.7; N, 12.8. This amide was hydrogenated catalytically, yielding α-hippuryl-lysine-amide which was isolated as the strongly hygroscopic hydrochloride, m. p. 248°. *Anal.* Calcd. for $C_{15}H_{23}N_4O_3Cl$: C, 52.5; H, 6.8; N, 16.3. Found: C, 52.0; H, 7.0; N, 15.9.

The tryptic proteinase was prepared according to E. Waldschmidt-Leitz and A. Purr [*Ber.*, **62**, 2217 (1929)]. The preparation contained no dipeptidase, aminopeptidase, and no carboxypeptidase; however, protaminase probably was present (Table I).

In contrast to HCN-papain, which splits only one peptide bond, tryptic proteinase splits two. After a complete splitting, hippuric acid was isolated from the digest (over 70% of the theoretical amount). Therefore, the splitting also must have liberated lysine and ammonia. That the free ε-amino group is an essential condition for the enzymic hydrolysis is shown by the fact that the above mentioned α-hippuryl-ε-carbobenzoxy-lysine amide is not hydrolyzed under the conditions of our experiments. The hydrolysis of our substrate by tryptic proteinase is remarkable since tryptic proteinase is supposed to react exclusively on anionic substrates.

TABLE I

ENZYMIC HYDROLYSIS OF α-HIPPURYL-LYSINE-AMIDE AT 40°

(Titration of liberated carboxyl groups)

Enzyme	Time, hrs.	Hydrolysis in % of one peptide bond
Tryptic proteinase, *p*H 8.8	22	123
	72	200
Tryptic proteinase, *p*H 8.8	18	121
	42	175
HCN-Papain, *p*H 5.0	5	58
	24	80
	49	85

It is intended to continue this research by studying the action of pure tryptic proteinases.

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MAX BERGMANN
WILLIAM F. ROSS

RECEIVED JULY 10, 1936

STEROLS. VI. SYNTHETIC PREPARATION OF OESTRONE (THEELIN)

Sir:

The evidence for the accepted structure of oestrone has recently been reviewed [L. F. Fieser, "Chemistry of Natural Products Related to Phenanthrene," A. C. S. Monograph Series, No. 70]. We have been able to prepare a well crystallized compound from ergosterol which by analysis, derivatives and mixed melting points, is identical with oestrone isolated from pregnancy urine. It has been previously shown that ergosterol may be converted into 3-hydroxy-nor-allo-cholanic acid [Chuang, *Ann.*, **500**, 270 (1933); Fernholz