Ethyl α,4-Dicyanocinnamate

p-Cyanobenzaldehyde was prepared by the aqueous copper nitrate oxidation of *p*-cyanobenzyl bromide using a method analogous to that of Moses[1] in 47% yield; 32 g. (0.25 mole) of this crude material, m. p. 97–98°, was dissolved in 200 cc. of isobutyl alcohol, together with 28 g. of ethyl cyanoacetate. When 0.5 ml. of piperidine was added, immediate warming took place and precipitation of the product started in about five minutes. After standing for four hours, the solution was cooled and filtered. The product was crystallized once from a mixture of ethanol and benzene and twice from ethanol alone; 50 g. (88%) of very pale yellow needles, m. p. 168.5–9 0° (uncor.) was obtained.

Anal. Calcd. for $C_{13}H_{10}O_2N_2$: C, 69.0; H, 4.46; N, 11.87. Found: C, 69.09, 69.27; H, 4.45, 4.68; N, 11.98.

CENTRAL RESEARCH LABORATORIES
MONSANTO CHEMICAL COMPANY
DAYTON, OHIO DAVID T. MOWRY

RECEIVED NOVEMBER 30, 1942

(1) Moses, *Ber.*, **33**, 2624 (1900).

COMMUNICATIONS TO THE EDITOR

NIACIN—NIACINAMIDE DIFFERENTIATION IN THE MICROBIOLOGICAL ASSAY PROCEDURE

Sir:

The microbiological niacin assay method of Snell and Wright[1] does not differentiate between niacin and niacinamide. Since these substances have equivalent vitamin activity, the inability to distinguish between them is ordinarily of no concern. In the case of tablets or solutions of niacinamide, designed for therapeutic use, however, the presence of any large proportion of the acid may be of interest. We have found a simple chemical reaction which specifically inactivates niacinamide, but is without action on niacin. It is the reaction usually called Hofmann's reaction,[2] *i. e.*, the action of bromine and potassium hydroxide upon amides. When applied to niacinamide, β-aminopyridine is formed,[3] which is apparently inactive microbiologically. We produce the reaction as follows.

To 1 ml. of a solution containing 1 mg. of niacinamide add 5 ml. of water and 1 ml. of bromine water (satd.), followed by 3 ml. of 30% potassium hydroxide. Allow to stand at room temperature for twenty minutes and then steam for twenty minutes. Cool, add an excess of 10 *N* sulfuric acid (blue to congo red), then remove any excess of bromine by use of a 4% solution of sodium acid sulfite. Use an outside, starch–iodide indicator to determine the end-point. The table indicates the results obtained when niacin, niacinamide and a mixture of the two were treated as described above. Reaction volume in all cases was 10 ml. The final solutions after neutralization, etc., were assayed by the Snell–Wright procedure.

No.	Vitamin, mg.	Bromine water, ml.	Total assay (niacin activity), mg.
1	1 Niacinamide	0.4	0
2	1 Niacinamide	1.0	0
3	1 Niacin	1.0	1.0
4	1 Niacin plus 1 Niacinamide	1.0	1.0

It is apparent, therefore, that the proportion of niacin to niacinamide in a preparation may be determined by estimation of the vitamin activity before and after treatment with bromine and potassium hydroxide. The possible application of this reaction to a differentiation of the forms of niacin occurring naturally is being investigated.

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NEW YORK, N. Y. CHARLES N. FREY

RECEIVED MARCH 24, 1943

(1) E. E. Snell and L. D. Wright, *J. Biol. Chem.*, **139**, 675 (1941).
(2) A. W. Hofmann, *Ber.*, **18**, 2734 (1885).
(3) A. Pictet and P. Crépieux, *ibid.*, **28**, 1904 (1895).

β,β-DIMESITYLVINYL ALCOHOL

Sir:

It seemed probable that, in the production of α,α-diarylacetaldehydes by the dehydration of the corresponding hydrobenzoins, β,β-diarylvinyl alcohols were formed as intermediates and that, being unstable, they isomerized to the corresponding aldehydes. Such an isomerization would be expected to become more difficult with increase in the size and complexity of the aryl radicals and it seemed possible that extreme crowding

might prevent the migration of the mesityl radical.

As a test of this hypothesis hydromesitoin (I) and isohydromesitoin[1] were treated with dehydrating agents. Rearrangement occurred in the normal manner. The product, apparently β,β-dimesitylvinyl alcohol (II), was obtained in 60% yield; m. p. 128–129°.

Anal. Calcd. for $C_{20}H_{24}O$: C, 85.97; H, 8.30; mol. wt., 280. Found: C, 85.68; H, 8.54; mol. wt. (ebullioscopic in chloroform), 283.

I II

The new compound reacted rapidly with methylmagnesium iodide to yield a mole of methane, and readily formed an acetate, a benzoate and a methyl ether. Hydrolysis of the esters regenerated the original compound. The infrared absorption spectrum measured in carbon tetrachloride solution showed peaks at 2.77 and 2.84 μ,[2] confirming the presence of a hydroxyl group. The compound was remarkably stable to heat and was not attacked by oxygen. Oxidation with alkaline hydrogen peroxide converted it to dimesityl ketone.

From these data it is evident that the rearrangement product behaves toward chemical reagents as though it had the enol structure (II) and that it must be at least partially enolic in carbon tetrachloride solution.

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RECEIVED FEBRUARY 20, 1943

(1) Fuson, Horning, Ward, Rowland and Marsh, THIS JOURNAL, **64**, 30 (1942).

(2) The authors are indebted to Professor W. H. Rodebush and Dr. J. B. Patberg for the measurement and interpretation of the infrared spectrum.

(3) du Pont Fellow in Chemistry, 1942–1943.

CAFESTEROL. III.—THE SUPPOSED ESTROGENIC ACTIVITY OF CAFESTEROL

Sir:

Hauptmann[1] and co-workers and, independently, Wettstein and co-workers,[2] have shown that cafesterol has no estrogenic activity, in contradiction to Slotta and Neisser.[3] Recently, at the Buffalo meeting of the American Chemical Society on September 7, 1942, P. N. Chakravorty and M. M. Wesner stressed again the existence of such an activity. To clear up the question we tested cafesterol and oxcafestandiol A[1b] as well as several parts of the unsaponifiable fraction of coffee oil and the oil itself following the Allen–Doisy procedure,[4] as described by B. Zondek.[5] For each substance we used five rats. The following scheme shows details of the fractionation.

SCHEME

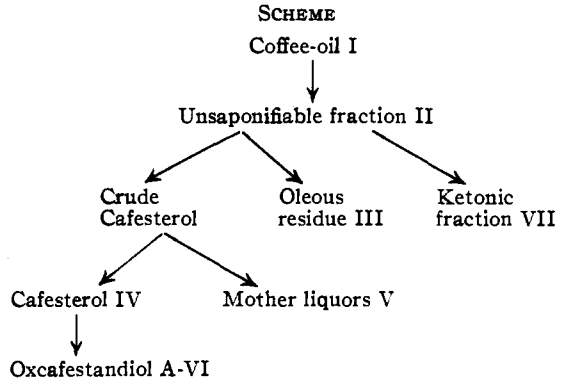

TABLE I

Fraction	Doses, mg.	Contains less R.U./g. than
I	2280	0.4
II	100	10
III	120	8.5
IV	30	33
V	117	8.5
VI	9	111
VII	36	29

None of the substances had estrogenic activity in the doses shown in the table (dissolved in 3 cc. of sesame oil). As 1γ of estrone always produced estrus, 1 g. contains 1,000,000 rat-units. Our substances certainly contain less rat-units in a gram than those indicated by the values (last column of the table) calculated from the non-efficient doses.

The experiments of Slotta and Neisser[3] were performed with mice. The mouse-unit is about one-fifth to one-tenth the rat-unit, which could possibly explain the discrepancies found by the various authors. Therefore we tested the whole unsaponifiable fraction (5 mg., 15 mg. was poison-

(1) (a) H. Hauptmann, P. Sawaya and L. Bruck-Lacerda, *Boletim da Faculdade de Filosofia, Ciencias e Letras da Universidade de S. Paulo*, Quimica nr. 1, 181 (1942); (b) H. Hauptmann and J. França, THIS JOURNAL, **65**, 81 (1943).

(2) A. Wettstein, H. Fritzsche, F. Hunziker and K. Miescher, *Helv. Chim. Acta*, **24**, 332E (1941).

(3) K. H. Slotta and K. Neisser, *Ber.* **71**, 1951 (1938). Memorias do Instituto Butantan, **11**, 71 (1938).

(4) E. Allen and E. A. Doisy, *J. Am. Med. Assn.*, **81**, 819 (1923); *Am. J. Physiol.* **68**, 138 (1924); **69**, 577 (1924).

(5) B. Zondek, *Klin. Wochschr.*, **8**, 2229 (1929).