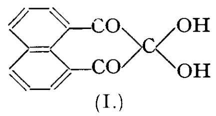
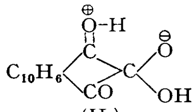
241. peri*Naphthindane*-1 : 2 : 3-*trione, and a Note on the Colour Reaction between its Hydrate and α-Amino-acids.*

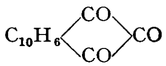
By Radwan Moubasher and (in part) William Ibrahim Awad.

*peri*Naphthindane-1 : 2 : 3-trione has been prepared by the action of thionyl chloride on its hydrate, or by pyrolysis of the latter. An inner-salt formula (II) for the hydrate is advanced, and some new reactions are described. The nature of the colour reaction between α-amino-acids and the hydrate is discussed.

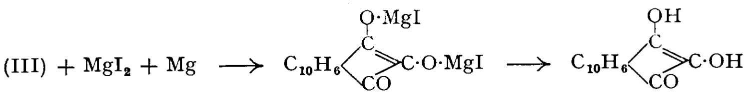
Errera (*Gazzetta*, 1913, **43**, I, 585; **44**, II, 18) heated *peri*naphthindane-1 : 2 : 3-trione hydrate (I) and obtained a red substance, believed to be the anhydrous compound (III), but he did not give an analysis. The latter can be prepared by heating (I) with thionyl chloride, or by heating it in a vacuum. The trione forms orange-red needles, which melt to a dark red liquid; on cooling, the red crystals are re-formed. When the red solution of the triketone in benzene is shaken with water, it is gradually decolorised owing to formation of the hydrate (I). The hydrate is readily soluble in cold water, and in order to explain this solubility and the remarkable thermal stability of (I), the inner-salt structure (II) is assigned to it. The possibility of resonance between (I) and (II) should be stressed (compare the inner-salt formula of ninhydrin, Schönberg and Moubasher, *J.*, 1943, 71).

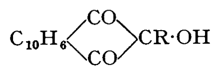

(I.)


(II.)


(III.)

Gomberg and Bachmann's reagent (*J. Amer. Chem. Soc.*, 1927, **49**, 2584) reduces *peri*naphthindane-1 : 2 : 3-trione to the dihydroxy*peri*naphthindenone (V), and the following mechanism is advanced:


(IV.) (V.)


(VI.)

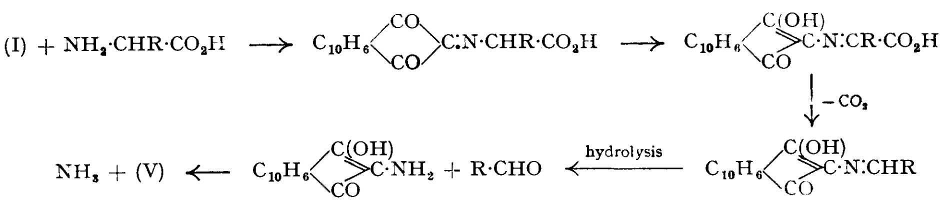
The hydrate (I) reacts with pyrrole, piperidine, and *p*-aminobenzoic acid giving the 2-*substituted* 2-*hydroxy*-peri*naphthindane*-1 : 3-*diones* of type (VI). This reaction is similar to that of bases, *e.g.*, aniline, and allied substances on (I) (Moubasher and Mostafa, *J.*, 1947, 130).

Further study of the reaction of the hydrate (I) with α-amino-acids has led to interesting results, for a deep pink colour is produced on warming a mixture of the two in aqueous solution. The reaction has been applied to many α-amino-acids, but appears to fail with β-amino-acids.

It gives, moreover, an intensely pink colour with blood albumin, egg albumin, and casein in alcoholic solutions, and a strong orange coloration is produced by normal human urine.

A pink colour is also obtained in presence of ammonia. This colour becomes violet and finally reddish-violet as the concentration of ammonia increases.

The reaction between ninhydrin and α-amino-acids has been discussed by Moubasher and Ibrahim (this vol., p. 702), and the reaction with the analogous *peri*naphthindane-1 : 2 : 3-trione hydrate follows a similar course, affording the compound (V) *via* several coloured intermediate stages :



EXPERIMENTAL.

Preparation of peri*Naphthindane*-1 : 2 : 3-*trione*.—(1) *By the action of thionyl chloride on its hydrate.* Freshly distilled thionyl chloride (50 c.c.) was heated on a steam-bath for about 40 minutes with the hydrate (0·5 g.) recently crystallised from water. The reaction vessel was connected by means of a ground-glass joint to a condenser fitted with a receiver connected to a filter pump, and the apparatus exhausted. The substance dissolved, forming an intensely red solution; as this became more concentrated (owing to evaporation of thionyl chloride), reddish crystals of *peri*naphthindane-1 : 2 : 3-trione separated. After about 50 minutes, the thionyl chloride had distilled off, and the vessel was placed in a vacuum desiccator containing sodium hydroxide in order to remove the last traces. The *trione* (yield, quantitative) melted at 265° (brown-red melt) (Found : C, 74·6; H, 3·2. $C_{13}H_6O_3$ requires C, 74·3; H, 2·8%).

(2) *By thermal decomposition.* The hydrate (0·5 g.) was heated in a vacuum at 250° (bath temp.) for ½ hour; it turned red and a sublimate of red needles was formed (m. p. 265°, brown-red melt), identical with those obtained in (1).

Properties of peri*Naphthindane*-1 : 2 : 3-*trione*.—It sublimes in a vacuum forming intensely red crystals. It dissolves in benzene, phenyl cyanide, xylene, or nitrobenzene at 100°, red solutions being formed. It is practically insoluble in light petroleum (b. p. 50—60°). It dissolves in concentrated sulphuric acid, giving an intensely violet coloration which changes to orange after some time. It gives with sodium hydroxide an intensely blue coloration.

Action of water. The trione was heated for about 5 minutes with a small quantity of water in absence of sunlight. When the hot solution was filtered, concentrated somewhat, and allowed to cool, yellowish crystals of the hydrate were obtained (yield, quantitative), m. p. 273° undepressed with an authentic specimen. When the trione was left at room temperature in May, it changed to the hydrate during 3 hours.

Action of Gomberg and Bachmann's reagent. To powdered magnesium (4 g.) in a mixture of dry ether (50 c.c.) and dry benzene (50 c.c., thiophen-free) iodine was added until its colour persisted. Dry magnesium powder (4 g.) was then added, followed by (III) (0·7 g.), and the mixture was shaken at room temperature for ½ hour. The product was added to ice-cold dilute hydrochloric acid and left overnight in an open vessel; the deposit was filtered off and crystallised from alcohol, whereby silky red needles (0·5 g.) of dihydroxy*peri*naphthindenone were obtained (identified by m. p., mixed m. p., and properties).

Reaction of peri*Naphthindane*-1 : 2 : 3-*trione Hydrate with Bases*.—(a) *Pyrrole.* The hydrate (1 g.), dissolved in water (50 c.c.), and pyrrole (1 g.), in 20 c.c. of alcohol, were mixed at room temperature and set aside; in 10 minutes the clear solution became turbid and a crystalline solid was deposited, and after an hour this was filtered off and recrystallised from alcohol, forming yellow needles of 2-*hydroxy*-2-1′-*pyrryl*peri*naphthindane*-1 : 3-*dione* (VI; R = C_4H_4N) (Found : C, 73·9; H, 4·2; H, 5·2. $C_{17}H_{11}O_3N$ requires C, 73·6; H, 3·9; N, 5·0%). It starts to decompose at 180° and melts at 200° (brown melt). It is soluble in alcohol or benzene, and gives a brown colour with concentrated sulphuric acid.

When this substance was heated under reflux with 10% sodium hydroxide solution (50 c.c.), a green coloration was first formed, changing to an intensely green-blue on cooling. The mixture was then acidified and cooled, whereby a crystalline colourless substance was produced; recrystallised from alcohol, this formed needles, m. p. 273°, identified (mixed m. p.) as naphthalic acid.

(b) *Piperidine.* The hydrate (1 g.) in 50 c.c. of water and piperidine (0·5 g.) in alcohol (10 c.c.) were mixed together at room temperature; after about ½ hour, the resulting yellow crystals were filtered off and recrystallised from alcohol, forming yellow needles (1·2 g.) of 2-*hydroxy*-2-1′-*piperidino*peri*naphthindane*-1 : 3-*dione* (VI; R = $C_5H_{10}N$), m. p. 150° (decomp.) (Found : C, 73·1; H, 5·7; N, 4·9. $C_{18}H_{17}O_3N$ requires C, 73·2; H, 5·7; N, 4·9%).

(c) p-*Aminobenzoic acid.* The hydrate (1 g.) in water (25 c.c.) and *p*-aminobenzoic acid (0·7 g.) in acetic acid (25 c.c.) were heated together on a steam-bath for 20 minutes; the resulting deep green crystals were filtered off and recrystallised from alcohol; 2-*hydroxy*-2-p-*carboxyanilino*peri*naphthindane*-1 : 3-*dione* (VI; R = $NH{\cdot}C_6H_4{\cdot}CO_2H$) formed yellowish-green needles (0·9 g.) decomposing at 185° and melting at 350° (Found : N, 3·9. $C_{20}H_{13}O_5N$ requires N, 4·0%).

Action of peri*Naphthindane*-1 : 2 : 3-*trione Hydrate on α-Amino-acids*.—On mixing a slightly warmed aqueous solution of the hydrate and glycine, an intensely pink colour is developed immediately, and the intensely red dihydroxy*peri*naphthindenone (V) is soon precipitated. All the other α-amino-acids tested react almost as rapidly as does glycine, and only mere traces are required : the hydrate in 10,000 parts of water gives the characteristic pink colour with α-amino-acids in 15,000 parts of water.

The shade of pink coloration produced shows but slight variations with the various α-amino-acids tested, *viz.*, glycine, DL-alanine, leucine (synthetic), DL-*iso*leucine, DL-norleucine, cysteine hydrochloride, aspartic acid, D-glutamic acid, phenylaminoacetic acid, phenylalanine, (—)-tyrosine, and DL-tryptophan.

This reaction is now being investigated with a view to the determination of α-amino-acids with the aid of a spectrophotometer.

Of the β-amino-acids, only β-aminopropionic and β-aminophenylpropionic acids have been tested, but they do not give any colour under the above conditions.

In the same dilution as above the hot reagent yields with dilute ammonia a strong pink coloration, but it is violet or reddish-violet with more concentrated solutions.

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