

## Electrophoretic Fractionation of the Various Subunits from Heat-Denatured Insoluble Collagen

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The collagen subunits are recognized in denatured soluble collagens<sup>1</sup> which have been fractionated either by chromatography in a carboxymethylcellulose column<sup>2</sup> or by gel electrophoresis.<sup>3</sup> Some commercial gelatins contain remnants of these subunits and certain extracts of insoluble collagen yield column chromatographic patterns which resemble those from soluble collagen.<sup>4,5</sup> We report here the gel electrophoretic demonstration of the various collagen subunits in the extracts from *insoluble* collagen.

The skins of adult guinea pigs (weighing in average 650 g) were cleaned from hair and subcutaneous fat, minced in a meat grinder and homogenized with a homogenizer of a revolving-blade type (No. 21 00 00, E. Bühler, Tübingen, West Germany) into cold 0.45 M sodium chloride. The neutral salt-soluble collagen was removed by three subsequent extractions with the 2-fold volume of 0.45 M NaCl overnight.<sup>6</sup> The supernatants were removed by centrifugation in MSE refrigerated centrifuge (17 000 rev./min for 120 min). The acid-soluble collagen was removed analogously with nine extractions under shaking with 0.15 M, pH 3.7, citrate buffer.<sup>7</sup> All preparations were carried out in a cold room (+ 4°C).

The insoluble residue was suspended in 0.01 M, pH 4.8, acetate buffer and shaken overnight. The supernatant was removed and the procedure repeated several times, until the supernatant did not foam any more, and its pH remained at 4.8.

The residue was suspended in the 4-fold volume of the 0.01 M, pH 4.8, acetate buffer and gelatinized at + 40°C for 15 min. The supernatant was collected by centrifugation at room temperature and analyzed with starch gel electrophoresis as described previously.<sup>3,8</sup>

The result is shown in Fig. 1. The regular subunits are well preserved, but the  $\beta$ -band and "x"-bands (presumably

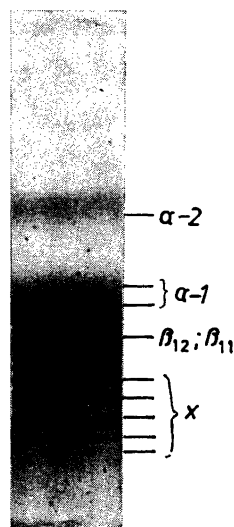


Fig. 1. Starch gel electrophoretic pattern of an extract (15 min at + 40°C) from insoluble collagen of guinea pig skin. The resolution of the  $\beta$ -components cannot be observed in this case.

larger aggregates) dominate. In these figures the splitting of the  $\alpha$ -1-band can be observed which has also been found in the samples prepared from acid-soluble collagen (V. Nántö, unpublished work from our laboratory).

Insoluble collagen consists thus of two fractions: (1) the first is solubilized as more or less intact subunits, when the secondary structure is denatured, and (2) the second is solubilized as smaller fragments only when the primary structure also is destroyed at higher temperature.

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## The Molecular Structure of Three Hydroxyquinones

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The crystal structures of nitranilic acid hexahydrate, chloranilic acid and ammonium chloranilate monohydrate have been determined by X-ray methods.

Table 1 gives the formulae, space groups, unit cell parameters, calculated and observed densities, and the number of formula units ( $Z$ ) per unit cell for the three substances. At the bottom of the table the number of observed structure factors and the reliability index are also given.

The chemical formulae of Table 1 are all written in quinoidal form whether this is supported by our experiments or not.

The structures were determined by standard methods and refined by least squares (diagonal) with isotropic temperature factors only.

In Fig. 1 the molecular structure of the three substances are given together with the molecular structure of the nitranilate ion previously reported by Jensen and Andersen.<sup>1</sup> From the figure it may be seen that the ring system of three of the compounds, namely nitranilic acid, nitranilate and chloranilate are much alike. Due to the center of symmetry present in all the molecules there are only three carbon-carbon distances to be discussed.

The three compounds are clearly not quinoidal, since they have two rather short carbon-carbon distances ( $C_1-C_2$ ) and ( $C_1-C_3$ ) of the same length, and one very long carbon-carbon bond ( $C_2-C_3$ ). Further-

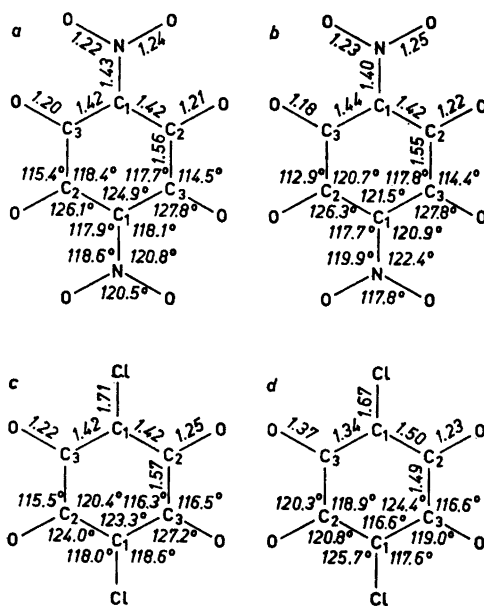


Fig. 1. Atomic distances (Å) and angles (°) in a: nitranilic acid from hexahydrate; b: nitranilate ion from ammonia salt; c: chloranilate ion from ammonia salt; d: chloranilic acid.

more the carbon-oxygen distances are equal (within our present accuracy).

Chloranilic acid, on the other hand, is in the quinoidal form. It has the same ring system as found by Robertson<sup>2</sup> and Trotter<sup>3</sup> in benzoquinone, and it has two kinds of carbon-oxygen distances. The shorter distances correspond to two carbonyl groups and the longer ones to C—OH groups (hydrogen has not yet been located). We had expected the nitranilic acid molecule to be in the quinoidal form also, the results however do not support our expectations. The acid is very strong ( $pK_I = -3.5$  and  $pK_{II} = -0.5$ ) and it is probably dissociated into nitranilate and oxonium ions in the solid hexahydrate. This proposal needs further experimental support which we hope to gain by location of the hydrogen atoms. All the molecules are planar except for the nitro group. This is twisted around the C—N bond so that the attached oxygen atoms are out of the ring plane.