The ultra-violet absorption spectrum is typical of the other isomers[2]; the rotation is lower, $[\alpha]_D^7 - 5{\cdot}4°$ (50 per cent alcohol); m.p. 177–179° (dec.). The crystalline nature was further shown by its characteristic X-ray pattern. *Analysis:* calc. for $C_{16}H_{18}O_9$: C, 54·2; H, 5·12. Found: C, 54·2; H, 5·44. Neutral equivalent, using as indicator the yellow-green colour formed on neutralization by base, 367; calc., 354. A similar titration with chlorogenic acid hemihydrate gave a neutral equivalent of 366 (calc. 363).

Saponification of 12·8 mgm. of neochlorogenic acid with sodium hydroxide in a nitrogen atmosphere allowed the isolation of 6·5 mgm. (91 per cent) of caffeic acid hydrate, m.p. 178–180° (uncorr.). After recrystallization from water, it was shown by mixed melting point and X-ray pattern to be caffeic acid. Quinic acid was also isolated, in poorer yield, from the non-ether soluble fraction of the hydrolysis products. It, too, was identified by mixed melting point determination and comparison of its X-ray pattern with that of an authentic specimen.

The position of attachment of the caffeoyl group to the quinic acid is not known.

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A Fluorescence Test for Serotonin and Other Tryptamines

The current interest in naturally occurring tryptamines, and the recent report by Shepherd *et al.*[1] on the detection of serotonin (5-hydroxytryptamine), prompt us to describe a more sensitive and specific test which has been used for some time in our investigations on the pain-producing agent of burn-blister fluid[2].

This test, which appears to be specific for those tryptamines which do not carry substituents at position 2 or at either of the nitrogen atoms, nor with heavily substituted side-chains, is performed thus. The solution under investigation, suitably diluted, is either spotted on to filter paper or, better, subjected to paper chromatography in an appropriate solvent system. The paper is dried in air, treated with a solution of ninhydrin in acetone (0·2 per cent) containing 10 per cent v/v of glacial acetic acid (spray or dipping technique[3]), the acetone allowed to evaporate, and the paper heated in an oven at 90–100° for 2–3 min. After heating, the paper is examined in ultra-violet light (3650 Å.); an area of intense blue-green fluorescence indicates a susceptible tryptamine.

The sensitivity of this test is such that 10^{-4} μmole/sq. cm. can readily be detected on a chromatogram; this corresponds to less than 0·02 μgm. of serotonin and is far beyond the sensitivity or specificity of the simple ninhydrin or Ehrlich colour reactions, or the fluorescence tests of Shepherd *et al.*[1] or of Udenfriend *et al.*[4]. Molar proportions of all the reacting tryptamines give very similar intensities and colours of fluorescence. The results shown in the table were obtained with tryptamine salts tested at a density on paper chromatograms of 10^{-3} μmole/sq. cm.

Tryptamine substituents	Appearance after treatment	
	Colour in visible light	Fluorescence in ultra-violet light
None (tryptamin)	—	bright blue-green
5-Hydroxy (serotonin)	—	bright blue-green
1-Methyl	strong pink, slowly fading	pale green after visible colour fades
2-Methyl	—	none
α-Methyl	—	bright blue-green
α-Ethyl	—	bright blue-green
α : α-Dimethyl	—	very faint golden
N : N-Dimethyl	—	none
α-Carboxy (tryptophan)	—	none

No fluorescence is given by tryptophan at any concentration. Furthermore, the purple ninhydrin colour from tryptophan or other amino-acid will not obscure the characteristic fluorescence of much smaller amounts of tryptamines mixed with the amino-acid. More than a hundred other amines, amino-acids and indoles have been tested, but none gives the characteristic fluorescence. The test works equally well on all types of filter-paper, including a specially purified paper, 10/*S*, supplied by Messrs. J. B. Green, Ltd., and with chromatograms run in acidic, basic or phenolic solvents (though the latter tend to destroy serotonin). The intensity of fluorescence is much reduced unless acetic acid is present in the ninhydrin reagent; this may explain why the great sensitivity and selectivity of this reaction was not noticed by Harris and Pollock, who first mentioned it[5].

The high-intensity fluorescence of the products of the reaction suggests that they are β-carboline derivatives. This view is supported by the negative results with certain substituted tryptamines. Substitution of the side-chain NH_2— would prevent condensation with a carbonyl compound; substitution at position 2 would prevent ring-closure of the condensed side-chain; di-substitution (but not mono-substitution) at the α-carbon atom would prevent dehydrogenation to the carboline; and many carbolines substituted at the indole-nitrogen have diminished fluorescence. Further investigations on the mechanism and specificity are in progress, but in the meantime the test should prove useful for studying the tryptamines in natural materials[2,4,6].

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