CHAPTER — II

CHARACTERISATION OF THE ANTITHIAMINE FACTOR ISOLATED FROM *Phaseolus radiatus*

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Physical and chemical properties of the antithiamine factor isolated from *Phaseolus radiatus* designated as compound 'G' were studied and summarised in Tables - 1 and 2.

Table - 1

PHYSICAL PROPERTIES OF COMPOUND 'G'

Properties	Observation
1. Colour	Light yellow
2. Texture	Micro crystalline substance
3. Solubility	Soluble in water, ether and easily soluble in ethanol, acetone and ethyl acetate
4. Stability	Heat-stable
5. Dialysis	Dialysable
6. Behaviour to litmus paper	Acidic
7. UV absorption maxima	300 nm

Table - 2

CHEMICAL PROPERTIES OF COMPOUND 'G'

Experiment	Observation	Inference
1. αα'-dipyridyl ferric chloride solution	Rapid formation of red colour	Presence of reducing group
2. Bromine in chloroform treatment	Slow decolorisation of colour	Presence of unsaturation
3. Treatment with sodium hydroxide	Intense yellow colour	May be due to presence of phenolic hydroxyl group

From the above physical and chemical properties of compound 'G', the presence of unsaturation and phenolic hydroxyl group can be predicted.

Detection of elements

The compound 'G' was shown not to contain nitrogen, sulphur, halogen and phosphorus.

Micro-analysis

Micro-analysis of compound 'G' showed the following percentage of the constituents : Carbon - 60.83%, Hydrogen - 4.57% and Oxygen - 34.6%.

Comparative study of different antithiamine compounds

The antithiamine activity of different compounds so far isolated from different sources as determined by the method as mentioned earlier (102) is given in Table - 3.

Table - 3

Name of the compound	Reference	Antithiamine activity (µg of thiamine hydrochloride inactivated by 1 mg of the compound)
Compound 'G'	—	135.0
Compound 'X'	(96)	3.5
Fraction 'A'	(96)	26.5
Methyl sinapate	(102)	45.0
3,4-dihydroxy cinnamic acid	(102)	135.0
3,5 dimethoxy salicylic acid	(103)	20.5

From the table, it appeared that antithiamine activity of the isolated compound 'G' was identical with that of 3, 4-dihydroxy cinnamic acid.

To demonstrate further similarity, if any, of the compound 'G' with 3, 4-dihydroxy cinnamic acid, various physico-chemical properties of compound 'G' and 3, 4-dihydroxy cinnamic acid were studied further under different conditions which are summarised below.

Melting point

Melting point of compound 'G' was found to be 191° C which was essentially identical with that of known standard 3, 4-dihydroxy cinnamic acid (97). Mixed melting point of the compound 'G' and that of known 3, 4-dihydroxy cinnamic acid mixture was also noted to be 192° C.

Thin layer chromatography

The identical nature of compound 'G' and 3, 4-dihydroxy cinnamic acid was verified by silica gel G thin layer chromatography using the following solvent systems:

I. Acetone : methanol =50 : 50 (v/v)

II. n-butanol : acetic acid : water = 80 : 10 : 10 (v/v/v) (Upper phase)

III. Chloroform : methanol : water = 60 : 20 : 20 (v/v/v).

After exposing the plates to iodine vapour followed by sulfuric acid charring test, spot for compound 'G' was found at a distance from base line which was essentially identical not only with known 3, 4-dihydroxy cinnamic acid but a mixture of compound 'G' and 3, 4-dihydroxy cinnamic acid in case of every solvent system. Thus, R_f values of compound 'G', 3, 4-dihydroxy cinnamic acid and a mixture of compound 'G' with 3, 4-dihydroxy cinnamic acid were found to be 0.70 in case of solvent system I, 0.73 in case of solvent system II and 0.91 in case of solvent system III. The chromatograms were shown in Figures - 6, 7 and 8. From this experiment it can be suggested that the isolated compound 'G' might be identical with 3, 4-dihydroxy cinnamic acid.

UV absorption spectral study

The UV absorption spectrum of compound 'G' was practically the same as that of 3, 4-dihydroxy cinnamic acid. Absorption maxima for compound 'G' was found to be at 300 nm which was identical to that of commercial 3, 4-dihydroxy cinnamic acid.

Infra-red absorption spectral study

To prove conclusively that the isolated compound 'G' was nothing but 3, 4-dihydroxy cinnamic acid, infra-red spectra of both compound 'G' and 3, 4-dihydroxy cinnamic acid in mujol were studied in the same paper using Parkin Elmer (model-137) infrared spectrometer. The profile was shown in Figure - 9. The IR spectra showed that curves for 3, 4-dihydroxy cinnamic acid and that of isolated compound 'G' were superimposeable with each other. Both the two compounds showed strong peaks at 3420 cm^{-1} (for phenolic hydroxyl group), 3250 cm^{-1} (for hydrogen bonded hydroxyl group), 1650 cm^{-1} (for carbonyl of carboxylic acid group), 1630 cm^{-1} (for aromatic and conjugated double bond).

FIGURE - 6

Silica gel G thin layer chromatographic experiment using solvent system I, i. e. Acetone : methanol = 50 : 50 (v/v)

FIGURE - 7

Silica gel G thin layer chromatographic experiment using solvent system II,
i. e. n-butanol : acetic acid : water = 80 : 10 : 10 (v/v/v, upper phase)

FIGURE - 8

Silica gel G thin layer chromatographic experiment using solvent system III, i. e.Chloroform : methanol : water = 60 : 20 : 20 (v/v/v).

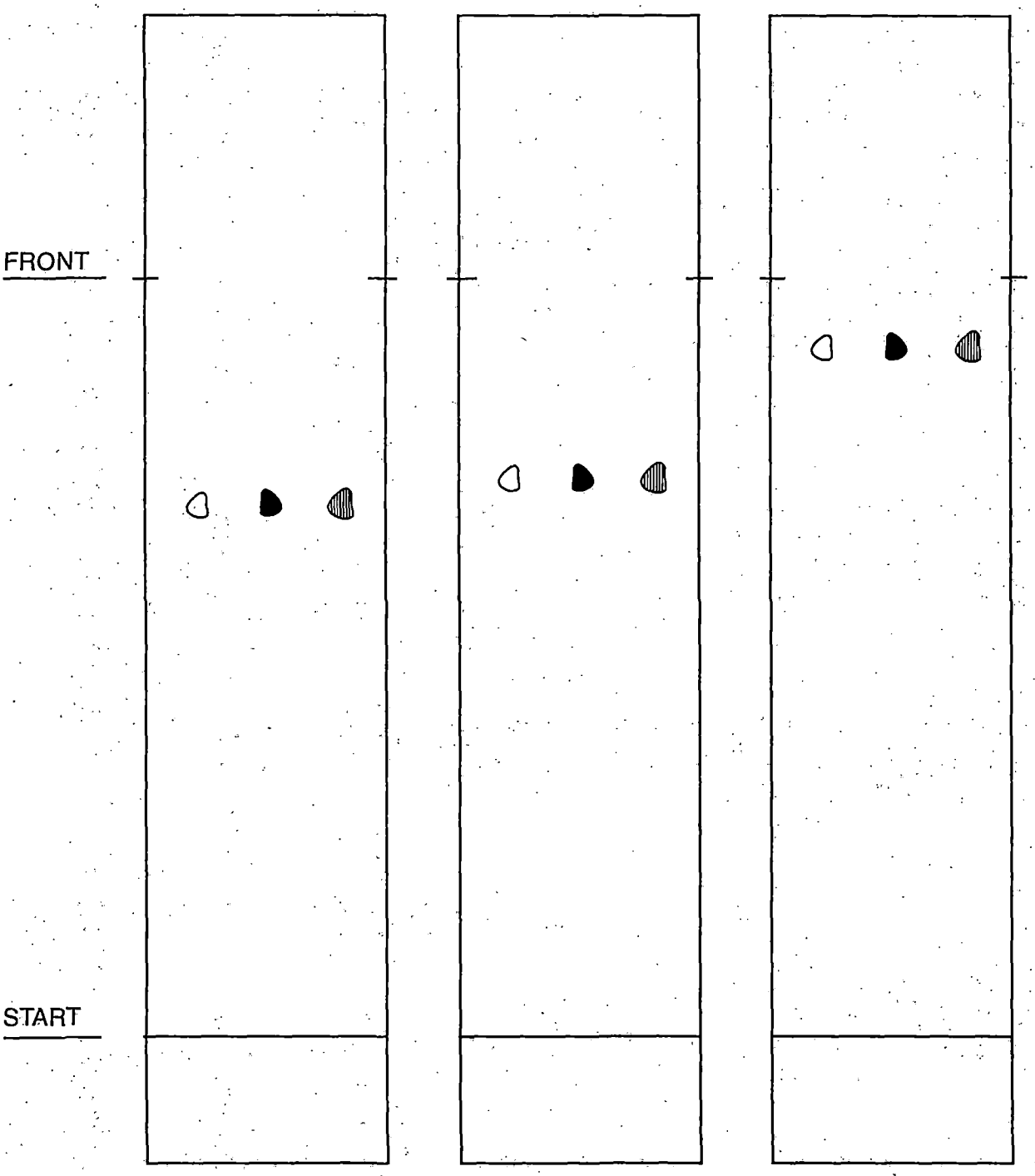


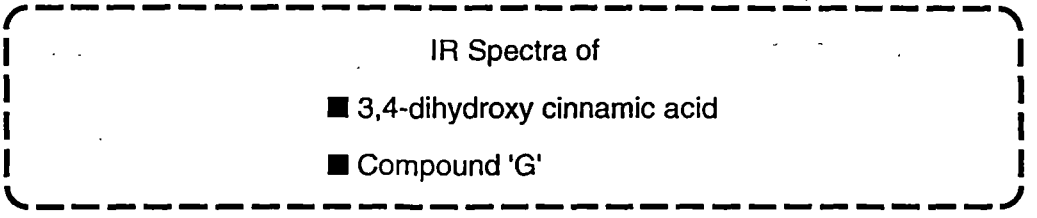
FIGURE - 6 FIGURE - 7 FIGURE - 8

COMPOUND 'G' —

3,4-DIHYDROXY CINNAMIC ACID —

MIXTURE OF COMPOUND 'G' & 3,4-DIHYDROXY CINNAMIC ACID —

FIGURE - 9



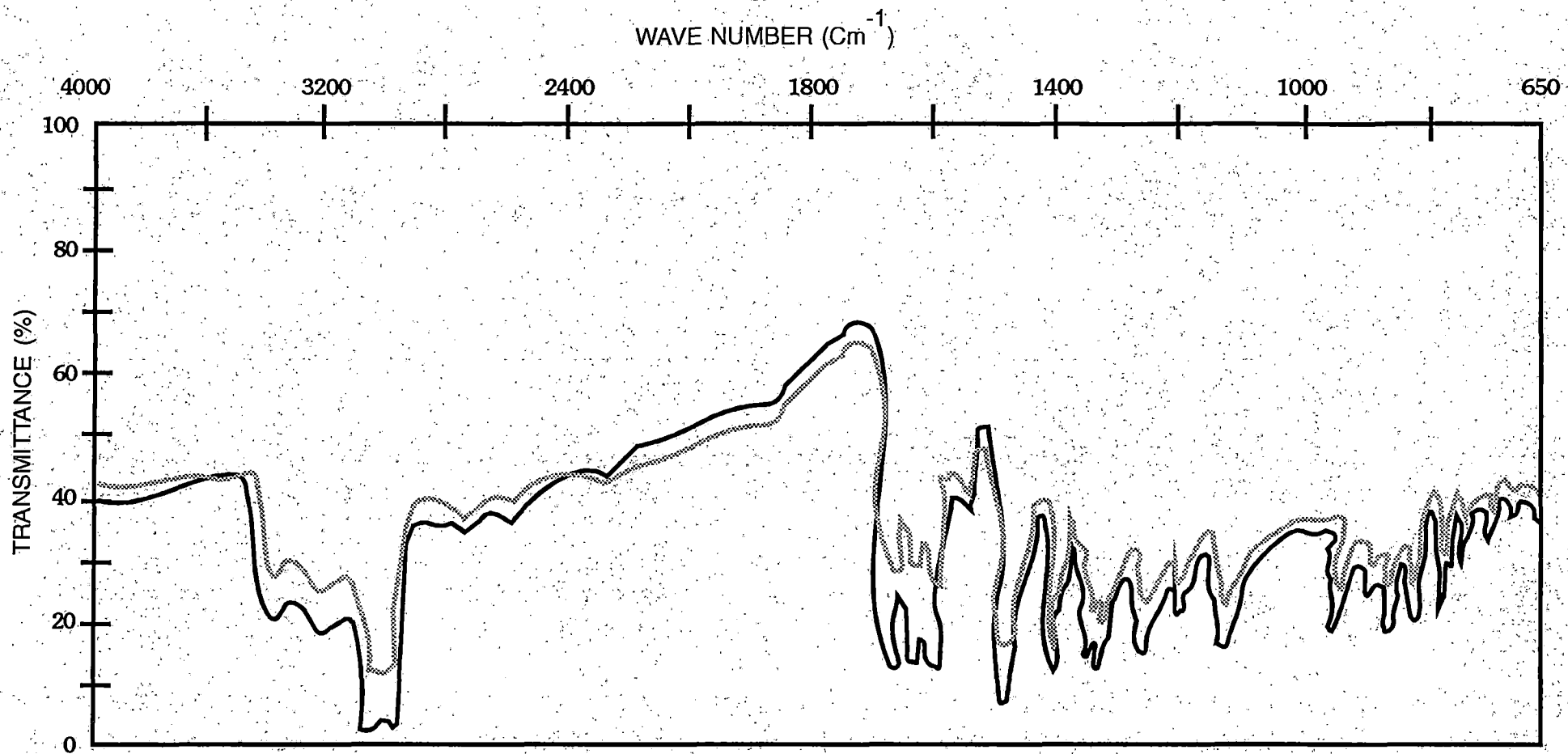
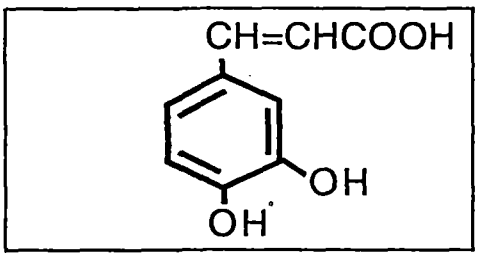

WAVE NUMBER (Cm -1)
4000
3200
2400
1800
1400
1000
650
100
80
60
40
20
0
TRANSMITTANCE (%)
: 3,4- DIHYDROXY CINNAMIC ACID
: COMPOUND 'G'

FIGURE - 9

Thus, the antithiamine factor isolated from *Phaseolus radiatus* and designated as compound 'G' possessing high antithiamine activity was found to be 3, 4-dihydroxy cinnamic acid.



3, 4-dihydroxy cinnamic acid.

(Compound 'G'.)