Evaluation of Curcumin

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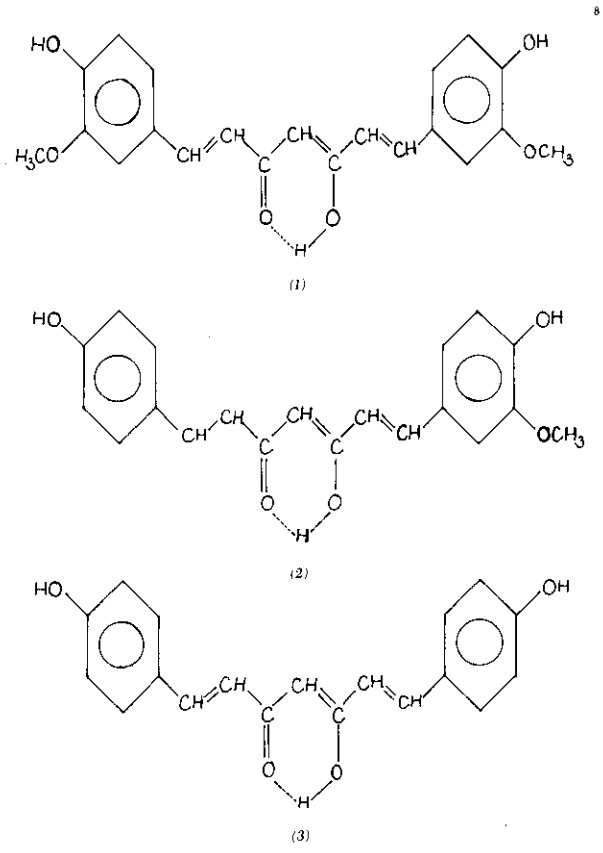
Curcuminoids confer tinctorial power to Curcuma longa L and other Curcuma species. Srinivasan analyzed this as principally curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione] *(1)* mixed with related demethoxy curcumin [4-hydroxycinnamoyl(4-hydroxy-3-methoxy cinnamoyl)methane] *(2)* and bis-demethoxy curcumin [bis-(4-hydroxycinnamoyl) methane] *(3)* and probably other minor constituents.[1] Synthesis as well as spectral data are in accord with these structures.[2-5]

Numerous methods are available for isolating curcumin from Curcuma longa L[6-10] and from other species.[11,12] Technical synthesis of this colorant is also known.[13] Except by the chromatographic route,[1,3,5] all others generally converge on curcuminoids with curcumin as the dominant constituent.[14(a),15,8] Purification of curcumin via the lead salts[15] is under study.

A recent recommendation resorts to mixed melting point determination with authentic specimen to check the purity of curcumin.[14(b)] However, firstly, the isolation of pure curcumin is very difficult and time-consuming and it is not produced commercially.[16(a)] One has to accept with caution the statement that "the industry wants pure curcumin and this know-how is also available to Indian manufacturers."[16b] According to Govindarajan, "pure curcumin which was not generally available in the early years ... is available now"[14(b)]; apparently, the author had in mind the curcumin of Eastman Kodak Co.[17] Secondly, melting point versus purity pattern is very confusing. For example, melting point



180-181° is recorded for Eastman Kodak Co. curcumin of 90.1% purity (Assay UV-VIS)[18] (vide infra) as against melting point 172-175° for E. Merck curcumin of 97% purity (Assay Alkalimetric).[19]

E. Merck has developed the alkalimetric

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method (Code No. AZL 0123480) for evaluating curcumin. A precisely weighed quantity of the substance (approx. 150 mg) is dissolved in approx. 50 ml of dimethyl formamide. Dimethyl formamide is titrated with 0.1N tetrabutylammonium hydroxide against azo violet before the addition of the substance. The solution of the substance is titrated to the end point potentiometrically under nitrogen.

1 ml of 0.1N TBAH = 18.4185 mg of $C_{21}H_{20}O_6$

Sastri has listed the divergent melting points of curcumin obtained from different sources.[8]

Janaki and Bose claim to have obtained chromatographically pure curcumin, mp 182-183°, by extracting in a Soxhlet apparatus the rhizomes of turmeric (species not specified) with petroleum ether, followed by benzene; the benzene percolate is then evaporated and residue crystallised from ethanol.[9]

On the other hand, Roughley and Whiting by similar processing (crystallisation from ethanol is not mentioned) of the rhizomes of Curcuma longa L obtained an orange powder which upon TLC examination showed the presence of three compounds *(1)*, *(2)*, and *(3)*.[3]

We owe to Srinivasan the step-wise use of petroleum ether and benzene for isolation of curcuminoids.[1]

It is almost certain that the curcumin (mp and mixed mp with BDH curcumin, 181-182°) secured by Khalique and Amin, by steam distilling the rhizomes of Curcuma longa L then extracting the residue with benzene and repeatedly recrystallising the solids from benzene extract using the same solvent, cannot be regarded as pure.[10] Typical assay (spectrophotometric) of BDH curcumin (No. 20031) indicates mp 174°, purity 85.7% (specification data of BDH Chemicals Ltd.).

Perhaps the most popular method for assaying curcumin is that of the American Spice Trade Association (ASTA).[20] For reasons which are not clear, Eastman Kodak Co. curcumin is specifically recommended as the standard in this estimation. Kalsec Inc.[21] and Norda International[22] have also accepted this curcumin as the base line in the spectroscopic methods for evaluating the dye.

To our knowledge, the purity of curcumin is best tracked by thin layer chromatography (TLC).[3,5] On submitting Eastman Kodak Co. 1179 curcumin, Lot No. A9B, to TLC with different solvent systems, the chromatograms exhibited at least three spots. Unquestionably, the curcumin is a mixture and this is corroborated by the disquieting disclosure in the lot analysis provided on specific request that the sample is only 90.1% pure![18] Such being the case, how ASTA can defend its position in recommending Eastman curcumin as a standard remains to be seen.

Following the Essential Oil Association (EOA) method[23] but measuring the absorbance at 425nm,[20] the colour values of different curcumin samples and therefrom curcumin percentage were determined against known standards; the results obtained are assembled in Table I. Referring to column 3, under a and b are given the percentage of curcumin calculated by taking Eastman curcumin as 90.1% and 100% pure respectively; under c and d are listed the percentage of curcumin relative to E. Merck and Synthite P curcumin* taken as 97.0%[19] and 100% pure, respectively. If the Eastman sample is 90.1% or 100% pure, the colour value of the E. Merck sample represents 100.29% or 111.32% curcumin, respectively! Compared to the E. Merck sample of 97.0% curcumin, Eastman and Synthite P curcumin amount to respectively 87.13% and 100.92% curcumin. On the other hand, if Synthite P curcumin is pegged at 100% purity, the percentage of curcumin in Eastman

Table I. % Curcumin from Color Value

Curcumin kind	Colour Value	E a	E b	% Curcumin c	% Curcumin d	Declared % Curcumin
Hopkin & Williams	13750	89.90	99.78	86.94	86.15	85.00[26]
Eastman Kodak Co.	13780	90.10	100.00	87.13	86.34	90.10
Synthetic (Prof.Whiting)	14850	97.09	107.76	93.90	93.04	-
E. Merck	15340	100.29	111.32	97.00	96.11	97.00
Synthite P	15960	104.35	115.82	100.92	100.00	-

*Strictly this dye should be designated as Synthite P curcuminoids.

and E. Merck samples sobers to 86.34% and 96.11%, respectively. Clearly then E. Merck and Synthite P curcumin are better standards than that of the Eastman curcumin.

Synthetic curcumin's colour value equalled only 93.04% curcumin based on Synthite P curcumin. Further work could not be done due to paucity of the dye.

It is believed that the colour value represents the integrated tinctorial strength stemming from "curcuminoids" as a whole. Though Synthite P curcumin registers a colour value amounting to ≧ 100% curcumin, TLC scanning (with various solvent systems) revealed it as a mixture of *1* and *2*. This is supported by its mass spectrum. Whereas M^+ 368 and 338 of *1* and *2* respectively are visible, M^+ 308 of *3* is missing. The molecular ion due to *2* happens to be the one derived from *1* by jettisoning CH_2O, a standard fragmentation pattern for the anisole system.[24] But in the mass spectrum of TLC pure *1*, there is no significant ion at M^+ 338.[25] One is therefore led to conclude that the signal M^+ 338 has originated from *2* present in Synthite P curcumin.

The question arises: What are the colour values of TLC pure curcumin and its associates? From the work of Roughly and Whiting[3] it is seen that $\log_{10} \epsilon$ of TLC pure, *1*, *2* and *3* at λ max EtOH: 430, 423 and 418 nm are 4.74, 4.71 and 4.57, equivalent to EOA colour values of 14916, 15130 and 12036, respectively. (The colour values of pure *1*, *2* and *3* calculated from UV absorption data of Kuroyamagi and Natori are respectively 14916, 10711 and 14473.)[5] One thing is certain, i.e., the colour value of pure curcumin is 14916.

Though TLC revealed the presence of *1* and *2* in Synthite P and *1*, *2* and *3* in the E. Merck curcumin, it is to be noted that in the light of above values, these samples have colour value exceeding that of pure *1*, *2* and *3* individually or collectively.

By what parameters are we then to judge turmeric extracts? If it is by their curcumin concentration, then there is a very severe limitation; pure curcumin, based on which this factor is determined, is not readily available. Are not turmeric extracts prized for their tinctorial power? EOA colour values, which can be measured independent of pure curcumin, reflect this quality fairly faithfully. Since Synthite P curcumin displays the highest EOA colour value of 15960, is it not justifiable to assess other turmeric extracts relative to this value? We therefore suggest that a colour value of 160 may be regarded as equivalent to one percent curcumin/oids. This will resolve contradictions that exist in the evaluation of turmeric extracts.

Experimental Section

General

Ultraviolet spectra measurements were recorded on Perkin-Elmer Spectrophotometer 551. Mass spectrum was obtained from MS-3074 Spectrophotometer. Colour values were ascertained by EOA method.[25] TLC was accomplished using Silica gel-G in the following solvent systems: (a) Methanol-benzene (10:1 v/v),[5] (b) Chloroform-ethanol (25:1 v/v),[3] and (c) Chloroform-benzene-ethanol (45:45:10 v/v).[27]

Materials: Standard Curcumin: Eastman Kodak Co. 1179 Lot No. A9B; E. Merck 820354; and Hopkin and Williams 355800 (C.I. 75300). *Synthite P Curcumin:* Turmeric dye (100 g: colour value 14300) derived from the rhizomes of Curcuma longa L is refluxed with ethylene dichloride (500 g) for 1 hour, filtered hot and the residue dissolved in hot ethylene dichloride (1250 g) and filtered. Partial removal of the solvent from the filtrate under vacuum gave a mate-

rial which upon multi-crystallisation from ethanol afforded the title compound: 29.5 g.

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