ISOLATION AND IDENTIFICATION OF CAPSAICIN AND ALLIED COMPOUND IN CHILLI

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ABSTRACT

The isolation and identification of capsaicin from fruits yielded two substances. The white crystalline compound, which, after U.V. and I.R. examination, exhibited the maximum absorption at 206 m\(\mu\) and the characteristics of long chain-\(n\)-alkyl, adjacent to a carboxyl group, was identified as palmatic acid. The molecular formula was, later on, confirmed to \(C_{19}H_{38}O_2\). The second, which was oily in nature, exhibited the presence of capsaicin due to its maximum absorption in U.V. at 230 and 281.5 m\(\mu\). It also represented all the properties of capsaicin oleoresin, when compared chemically as well as physically with the commercial oleoresin of capsaicin E. Merck Darmstadt, but could not be crystallized even after chromatography over neutral and acidic alumina with various solvents or after subjecting it to high vacuum distillation (10\(^{-4}\) mm).

I. INTRODUCTION

CHILLI (Capsicum annuum L.) is an important condiment grown for its fruits in the plains and hills of India. It is rich in capsaicin (Claus, 1961; Balbaa, 1968). Capsaicin, responsible for pungency in chilli is a colourless, crystalline compound and ranges from 0.14 to 0.22% in chilli varieties (British Pharmaceutical Codex, 1963). Methods for isolation, extraction and estimation of capsaicin have been modified from time to time (Lapworth and Royle, 1919; North, 1949; Karawya, et al., 1967). Sometimes allied compounds are also found. Therefore in this experiment a modified method for isolation and identification of capsaicin or its allied compound was studied.

II. MATERIALS AND METHODS

The method of isolation and identification, as described by Lapworth and Royle (1919) and Spath and Darling (1960), was very lengthy and time-
consuming. The same has, therefore, been modified to extract capsaicin easily and with more recovery.

After a number of preliminary experiments on solubility and related chemical properties, the following method was applied to isolate and identify the capsaicin or its allied compounds, if any.

1,250 gm of well-powdered chilli fruits were exhaustively soxhleted with solvent ether. The fruits extract, thus obtained, was extracted three to four times with 15 ml of 5 per cent KOH, which dissolved all the capsaicin in the alkaline solution.

The alkali solution containing capsaicin was divided in two parts. In one part CO₂ was passed till the solution had no colouration with phenolphthalein indicator, while in the other, sufficient quantity of NH₄Cl was added and slightly warmed. This process converted all KOH into ammoniacal form. Capsaicin was not soluble in both the solutions, either treated with CO₂ or NH₄Cl. Later on, these solutions were extracted separately with ether and the ether solutions were concentrated. Residues of the extracts were diluted with 50 mls methanol (norit) separately, filtered through Whatman 40 filter-paper and concentrated. The concentrates were kept in the cold with dilute methanol and the resulting crystalline substances were filtered under suction, and the crystallisates were dried under vacuum. The mother liquors were again treated with charcoal and some more of the crystalline compound could be obtained in the same manner. To get the substances in a pure, white and crystalline form, fractions collected from both the procedures, having similar melting points were mixed and recrystallised several times till there was no rise in the melting point. After final crystallisation the compound was purified by passing through neutral and acidic alumina columns.

The mother liquors, thus collected and freed from solvent, were oily in nature. This oily residue did not yield any crystalline compound even after chromatography over neutral and acidic alumina with various solvents or after subjecting it to high vacuum distillation (10⁻⁴) mm.

The crystalline compound was identified using U.V. spectrum, Infrared spectrum, melting point and microanalytical data. The mother liquor was also examined spectroscopically.
III. Results

The isolated pure, white and crystalline compound of 0.700 gm and 2.00 gm of oily mother liquor, which could not be crystallised, were examined for identification of capsaicin or its allied compound as given below:

**The Crystalline Compound**

*U.V. spectrum.*—In methanol, the compound shows the maximum absorbance at 206 mμ and the lowest at 227 mμ and 285 mμ (Fig. 1). This confirms that the compound under examination is other than capsaicin, which shows strong peaks at 230 mμ and 281.5 μ.

![Ultra-violet spectrum for the crystalline compound isolated from chilli fruits.](image)

Infra-red spectrum.—As shown in Fig. 2, the compound is optically inactive. In the infra-red regions, peaks are at 3000, 2500, 1700, 1435, 1296 and 940 cm⁻¹. These obviously indicate a carboxylic acid dimer, and the evenly spaced absorptions in the range 1350-1180 cm⁻¹ are characteristic of long-n-alkyl chains. Further, the band 1418 cm⁻¹ indicates a CH₂ grouping adjacent to a carboxyl group. The bands 733 and 725 cm⁻¹ indicate methylene chain rocking. The bands between 1350 and 1180 cm⁻¹ show long-chain-n-alkyl compounds and the evenly spaced bands are characteristic of chain length.
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According to the relation between the number of bands and the chain length, there were six bands with intervals of 20 cm\(^{-1}\). The compound thus appears to be palmitic acid which was confirmed through a mixed melting point determination as well as superimposable infra red spectra.

![Infra-red spectrum for the crystalline compound isolated from chilli fruits.](image)

**Fig. 2.** Infra-red spectrum for the crystalline compound isolated from chilli fruits.

**Melting point.**—The melting point of the compound remained constant between 56–57° C.

**Micro-analysis.**—The analytical data confirmed to the molecular formula \(C_{16}H_{33}O_2\).

**The Mother Liquor**

The mother liquor yielded 2.00 gm of oily substance. This was examined in U.V. spectra at 230 m\(\mu\) and 281.5 m\(\mu\) for the presence of capsaicin. The results obtained clearly showed the presence of capsaicin (Fig. 3) in strong concentration. This substance represents all the properties of capsaicin oleoresin and should, therefore, be considered as the oleoresin of capsaicin. The oleoresin was then tried for crystallisation from methanol and \(n\)-hexane, but the results were not conclusive and definite. The substance, after passing through acidic and neutral alumina columns and subsequent crystallisation, also gave unsuccessful results. It was further distilled in high vacuum (10\(^{-4}\) mm) and then tried for crystallisation, but the attempts were again not successful.
IV. DISCUSSION

The isolation and identification of capsaicin from fruits yielded two substances. The white crystalline compound, which, after U.V. and I.R. examination, exhibited the maximum absorption at 206 m\(\mu\) and the characteristics of long chain-\(n\)-alkyl, adjacent to a carboxyl group, was identified as palmatic acid. The molecular formula was, later on, conformed to \(\text{C}_{19}\text{H}_{38}\text{O}_2\). The second, which was oily in nature, exhibited the presence of capsaicin due to its maximum absorption in U.V. at 230 and 281·5 m\(\mu\). It also represented all the properties of capsaicin oleoresin, when compared chemically as well as physically with the commercial oleoresin of capsaicin E. Merck Darmstadt, but could not be crystallised even after chromatography over neutral and acidic alumina with various solvents or after subjecting it to high vacuum distillation (10\(^{-4}\) mm).

![Graph showing ultra-violet spectrum for the Oleoresin of capsaicin isolated from chilli fruits.](image)

**Fig. 3.** Ultra-violet spectrum for the Oleoresin of capsaicin isolated from chilli fruits.

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VI. REFERENCES


