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Study on the Natural Pigments Present in the Hulls of *Garcinia Mangostana* Linn

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Abstract

Garcinia mangostana Linn, (Family: Guttiferae) is known to be a rich source of bioactive molecules including flavonoids, benzophenones and lactones. A variety of xanthones have been isolated from mangosteen plants and fruits include the hull, rind heartwood and flesh. The fruit hull is used as an astringent and also used against cholera, dysentery and diarrhoea in traditional medicinal system.

Petroleum ether extraction of the dried and powdered fruit hulls followed by column chromatographic separation afforded two compounds, namely, gartanin (1) (0.48% in yield, mp 146-152°) and mangostin (2) (0.97 % in yield, mp 179-180°). The structures of these two compounds have been identified by UV, FT-IR, NMR and EIMS. These identifications were confirmed by comparison of melting point values with reported data in the literature.

Keywords : *Garcinia mangostana; Guttiferae; xanthones; mangostin; gartanin*

Introduction

Mangosteen, *Garcinia mangostana* L., (family: Guttiferae) is known as the "Queen of fruits". Its origin is in the Southeast Asia, probably the Malay Archipelago. It can be found in Northern Austrilia, Brazil, Myanmar, Central America, Hawaii, Southern India, Indonesia, Sirilanka, Thailand, Vietnam, and other tropical countries. Mangosteen is one of the most widely recognized tropical fruits and has universal appeal because of its quality in color, shape and flavor (Erickson, 2001). In Myanmar, the trees bear one crop in a year, during the monsoon; June - August.

The fruit hull of mangosteen has been used many years as a medicine for treatment of skin infection, wounds and diarrhea in Southeast Asia (Nakatani, 2002). Powdered rind has given satisfactory results in the treatment of tropical dysentery. The active principle appears to be a yellow pigment, mangostin. Clinical trials, indicate that mangostin is inferior to the powdered

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rind as in an anti-diarrhoeal agent (Wealth of India, 1956). Recent research reveals that mangostin showed potent inhibitory activity against HIV-1 protease (Chen, 1996). Polysaccharides from the pericarbs of mangosteen can stimulate phagocytic cells and kill intracellular bacteria (Chanarat, 1997).

Mangostin is the yellow colouring matter obtained from various parts of the mangosteen tree. It was isolated first by Schimid in 1855 from the fruit hulls. Mangostin is a member of the relatively small group of naturally occuring xanthones (Yates, 1958). The fruit hulls, bark and dried latex of mangosteen contain mangostin, $C_{24}H_{26}O_6$, m.p. 181-183°. The dried latex is a particularly rich source, yielding 30-50% of mangostin and a minor yellow colouring matter (2%), m.p. 175.5° which he named β-mangostin; $C_{25}H_{28}O_6$ (Yates, 1968).

Five tetraoxygenated xanthones:

garcinone A, garcinone B, garcinone C, mangostin and γ-mangostin have been isolated from $CHCl_3$ extract of the fruit hulls of *Garcinia mangostana* (Sen et al, 1981).

Five polyoxygenated xanthones:

mangostin, β-mangostin, nor-mangostin, gartanin and 8-desoxygartanin have been isolated from very ripe fruit hulls of mangosteen (Ashisen, 1980).

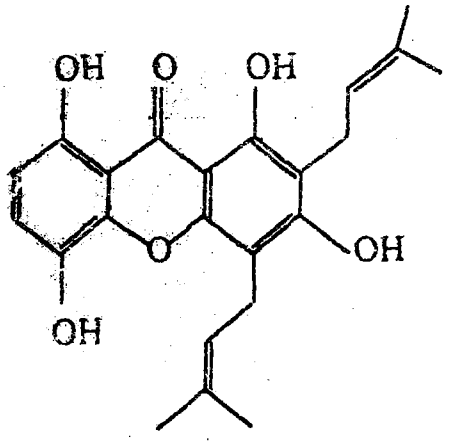
Three new xathones:

mangostenol, mangostenone A and mangostenone B from the green fruit hulls of mangosteen (Suksamram, 2002).

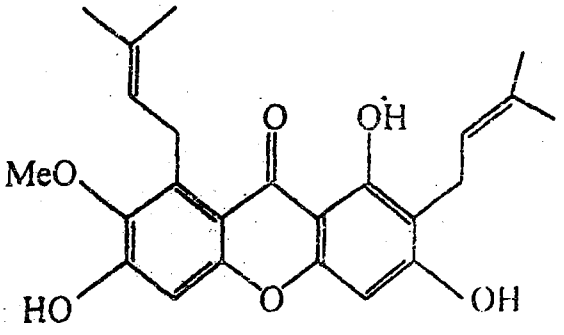
Biological activity:

Some chemical constituents isolated from mangosteen have shown different bioactivity, for example, mangostin is active in suppressing the 'acute' as well as the 'chronic inflammations; Alpha-mangostin inhibits methicillin-resistant *staphylococcus aureus*, MIC=1.6-12.5 μg/ml (Linuma, 1996). Garcinone E may be potentially useful for the treatment of certain types of cancer (Ho, 2003). Gamma-mangostin was found to be directly inhibit cyclooxygenase (COX) enzyme activity in rat glioma cells (Ohizumi, 2003).

Myanmar people traditionally employ a decoction of the hulls and bark as a febrifuge and to treat diarrhea, dysentery and urinary disorders (Yates, 1957). In addition, the chemical constituents present in mangosteen may depend on its origin and maturation of the fruit. Because of growing interest in correlating phytochemical constituents of a plant with its pharmacological activity (Gupta, 1994), it is essential to investigate the bioactivity and chemical constituents present in fruit hulls of mangosteen.



Gartanin (1). $C_{23}H_{24}O_6$



Mangostin (2). $C_{24}H_{26}O_6$

Materials and Methods

General procedures

Mps: uncorr; ^1H (400 MHz) and ^{13}C (100 MHz) NMR: BRUKER DPX-400, $CDCl_3$ with TMS as int. standard; UV: shimadzu UV-240, cyclohexane; FT-IR: Perkin Elmer GX system, KBr; GC-MS: Perkin Elmer GCMS; CC: Merck silica gel 60 (70-230 mesh), eluents: petroleum ether - ethyl acetate (PE-EtOAc), TLC: 0.25 mm precoated silica gel (60 F_{254}, Merck), solvent system: **I**. toluene : ethyl acetate (4:1) and **II**. ethyl acetate : pet ether (3:1), spots were detected by inspection under UV light (254 nm or 365 nm) or by the colour developed with 5% sulphuric acid spraying followed by heating.

Plant Materials

Mangosteen used in this study were collected from Kyeik-kaw in Mon State in June, 2003.

Preliminary Phytochemical Examination

Preliminary phytochemical examinations were done on this sample according to the standard methods as follows: Test for carbohydrate (Priestman, 1953 and Vogel, 1966); Test for glycosides (Chakavatic, 1952 and Trease, 1954); Test for organic acid (Vogel, 1956); Test for phenolic compounds (Vogel,1956); Test for reducing sugar (Finar, 1964); Test for Tannis (Linsted, 1955); Test for α-amino acid (Kirtaka, 1975 and Nedkarni, 1954); Test for alkaloids (Chakavatic, 1952, Rangaswami, 1955 and Trease, 1954); Test for steroids (Finar, 1964); Test for flavonoids (Finar, 1964) and Test for cyanogenic glycoside (Steece, 1949).

Extraction and Isolation of Compounds

Procedure I

The air-dired, powdered fruit hulls (100.0 g) were extracted with pet ether (60-80°) (600 ml) for 8 hours in a Soxhlet extractor. Removal of the solvent provided crude extract (1.00 g), which was chromatographed on silica gel (50 g, d = 2 cm, l = 20 cm) in pet ether. The column was eluted with increasing amount of EtOAc in PE; PE - EtOAc (9:1), PE-EtOAc (17:3), PE-EtOAc (4:1). 10 ml in each fraction were collected and the chromatography was monitored by TLC using **I** : PE-EtOAc (1:3) V/V; **II** : PE-EtOAc (3:1) V/V solvent system. The fractions that gave similar TLC pattern were combined together and concentrated. In this way seven combined fractions F_I to F_{VII} were obtained and used for further purification. Compound **1** (gartanin) was isolated from F_{III} after crystallization in benzene. It is yellow needles and the yield was 0.48 % (23.1 mg). Compound **2** (mangostin) was isolated from F_{IV} after crystallization from benzene. It is orange needles and the yield was 0.97 % (46 mg) based upon the crude extract.

Procedure: II

The dried powdered fruit hulls (100.0 g) were refluxed with petroleum ether 60-80 ° (3 times x 400 ml). The combined PE extract was concentrated to about 100 ml. On standing overnight, 1.25 g of yellow solid precipitated out and which was removed by filtration. Further standing of mother liquor at room temperature gave an additional 2.25 g of precipitate. Fractional crystallization was done for six times and the yield of total precipitate was 23.1 % (23.1 g). 1.0 g of Yellow precipitate (second crop) was then chromatographed on silica gel column (50 g, d = 2 cm, l = 20 cm) in pet ether. The column was eluted with increasing amount of ethyl acetate in PE; PE-EtOAc (9:1), PE-EtOAc (4:1), PE-EtOAc (3:1). A quantity of 5 ml was collected for each fraction. Fraction with similar TLC pattern were combined and concentrated. In this way, four combined fractions $\mathbf{F_A}$ **to** $\mathbf{F_D}$ were obtained. The fraction $\mathbf{F_C}$ was further purified by crystallization in PE-EtOAc (1:3) providing gartanin (**1**). Crystallization of first crop of precipitate provided pure mangostin (**2**).

Results and Discussion

Preliminary Phytochemical Investigation

Carbohydrate, glycoside, flavonoid, organic acid, phenolic compound, reducing sugar and tannin were found to be present. α-Amino acid, alkaloid, steroid, and cyanogenic glycoside were found to be absent.

Identification of Isolated Compounds

Chromatographic separation of PE extract and further purification provided two xanthones, gartanin (**1**) and mangostin (**2**). Mangostin was isolated as a major constituent from PE extract of *Garcinia mangostana*. The chemical and spectral data of two xanthones are shown as follows.

Gartanin (1)

$C_{23}H_{24}O_6$, yellow needles (23.1 mg, 0.48% yield); m.p 146°-152 °C (lit. 167 °C, Govindachari et al, 1971); λ_{max}(cyclohexane) 240, 260, 281, 350

nm (lit. 259, 284, 325 (sh), 351 nm, Govindachari et al, 1971); ν_{max} (KBr) 3419 (ν O-H), 2969 (ν C-H), 1628 (ν C=O), 1376 (δ CH_3) and 1284 (ν_{ar} C-O); δ ($CDCl_3$) 12.3 (1H, s), 11.3 (1H, s), 7.21 (1H, d, J = 9 Hz), 6.25 (1H, d, J = 9Hz), 5.4 - 5.2 (2H, m), 3.52 - 2.95 (4H, br), 1.80 (12H, s) and 1.70 (12H, s);δ_c 116.87(-CH=CH_2), 121.09 (aromatic C-H), 130.05 (aromatic C-C), 135.80 (-CH = CH_2), 142.95 (aromatic C-O-CH_3); EI - MS m/z (%) 396 (M+) 381, 353, 341, 325, 297, 285 (100%) (Lit. 396 (M+), 381, 353, 341, 325, 297, 285 (100%), 38/1, 379, 353, 341, 325, 297, 285).

Mangostin (2)

$C_{24}H_{26}O_6$, orange needles (46 mg, 0.97% yield); m.p 179-181 °C (lit. 181 °C., Govindacharia et al, 1971), λ_{max}(cyclohexane) 245, 261, 308, 353 nm (lit. 243, 259, 318, 351 nm, Yates et al, 1958); ν_{max} (KBr) 2928 (C-H), 1622 (C=C), 1185 (C-OH), 841 cm^{-1} (C-H); δ_H ($CDCl_3$) 13.7 (1H, s), 6.75 (1H, s), 6.35 (1H, s), 5.25 (2H, m) 4.1 (2H, d, J = 9 Hz), 3.25 (2H, d, J = 9Hz), 1.69-1.64 (3s, 12H); δ_c 62.065 (R-O-CH_3), 112.60 (aromatic C-H), 121.49 (aromatic C-H) 132.62 (aromatic C), 137.10 (-CH=CH_2); 142.70 (aromatic C-O-CH_3); EI-MS m/z (%) 410 (M+) 382, 367, 354, 339 (100 %), 311, 285, (lit. 410 (M+), 393, 367, 355, 354, 339 (100%), Wan, 1973).

Conclusion

From the present investigation, natural pigment - gartanin (0.48% yield, m.p 146 - 152°C) and mangostin (0.97% yield, m.p 179-180°C) have been isolated. The structures of the isolated compounds were elucidated by UV, FT-IR, IH and ^{13}C-NMR and EIMS Spectroscopic methods.

Acknowledgement

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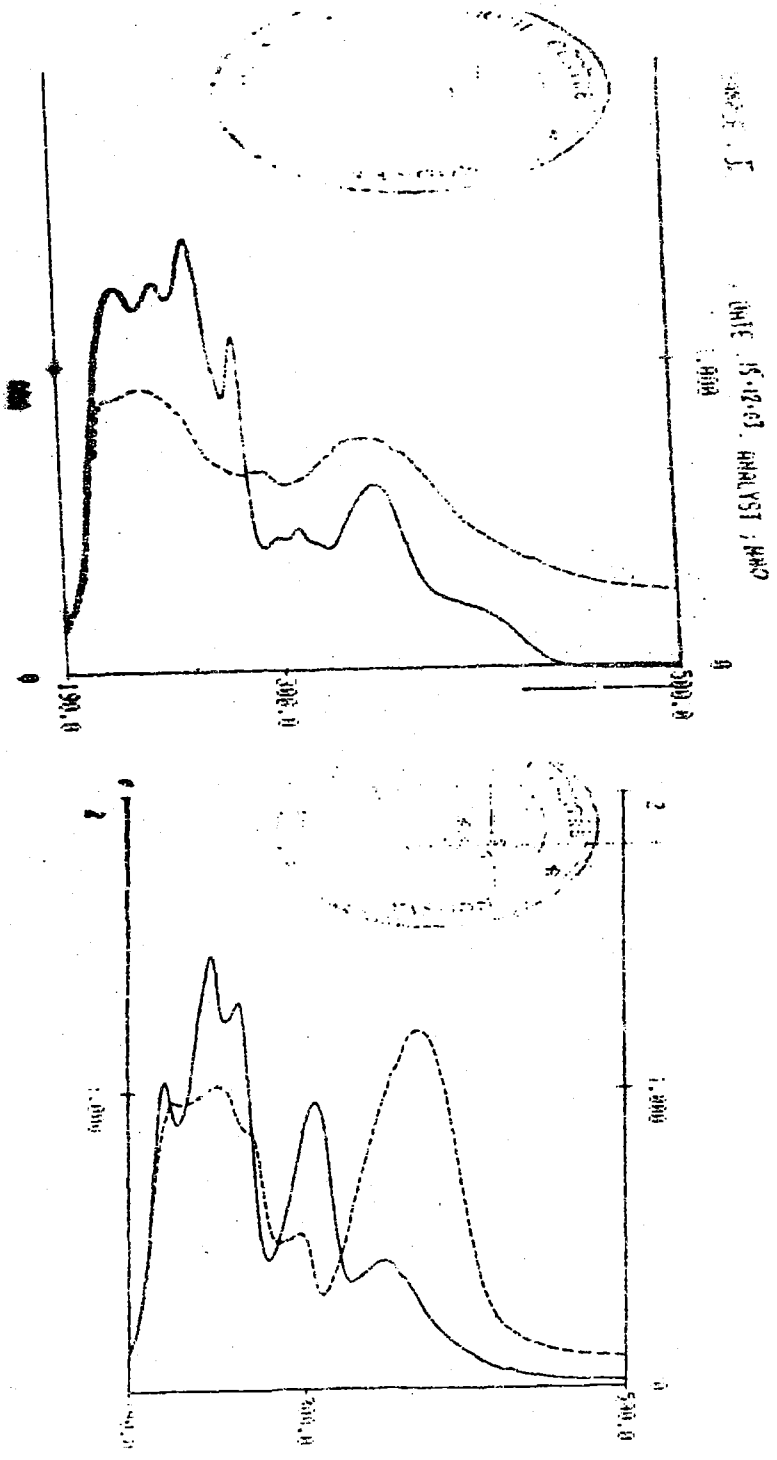

Figure 1. UV Spectra of isolated compounds (I and II)

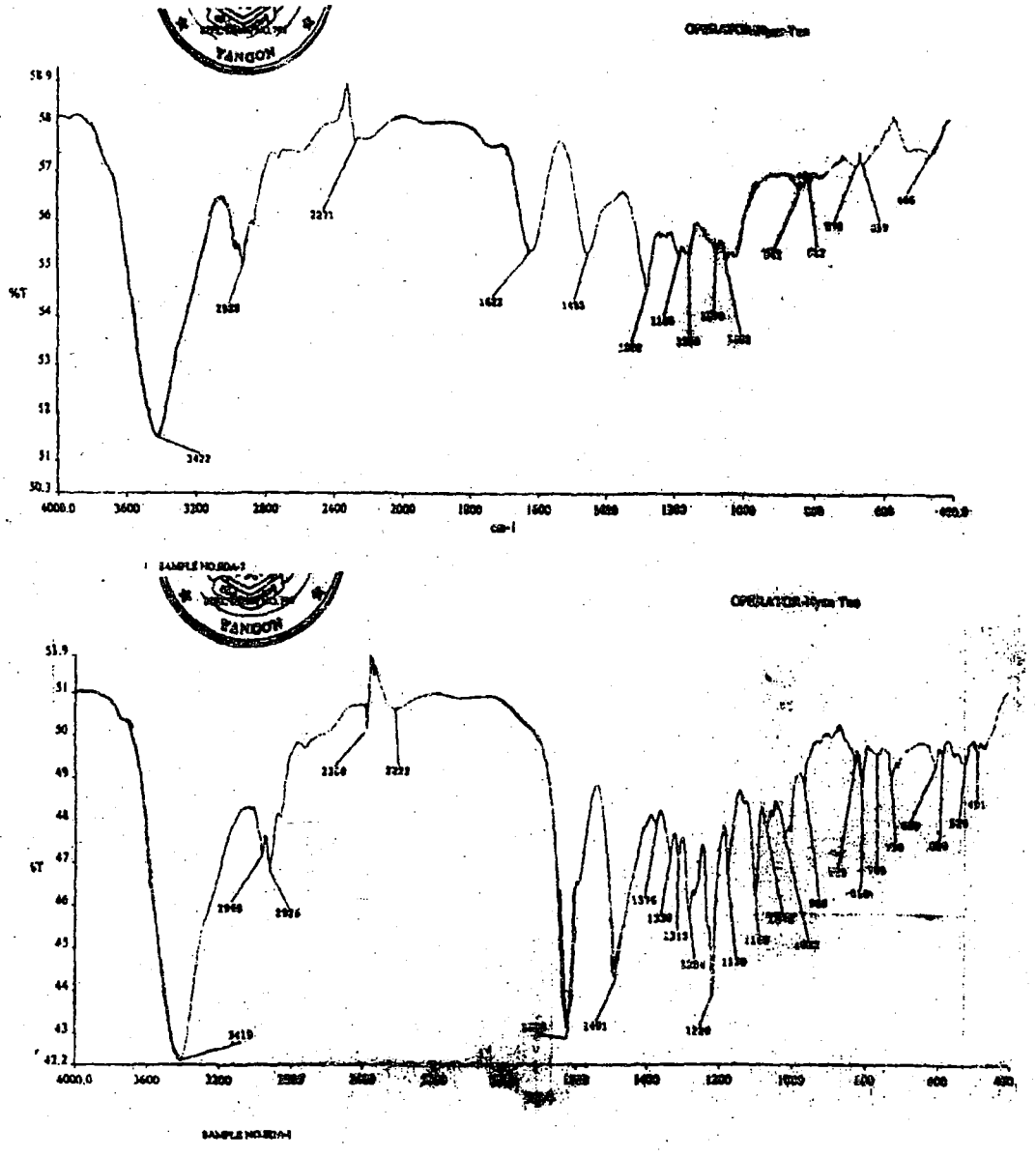

Figure 2. FT-IR Spectra of isolated compounds (I and II)

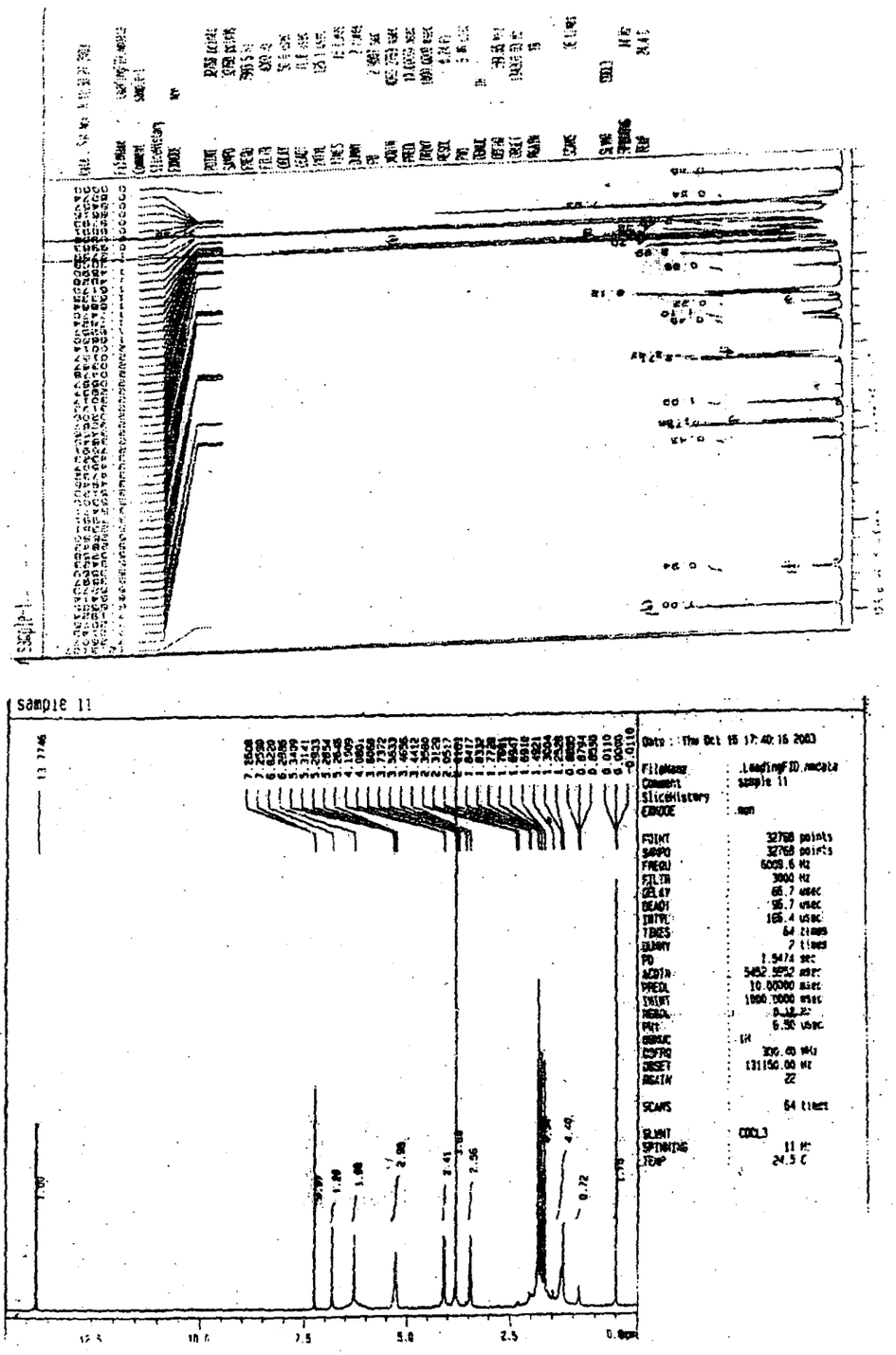

Figure 3. HNMR Spectra of isolated compounds (I and II)

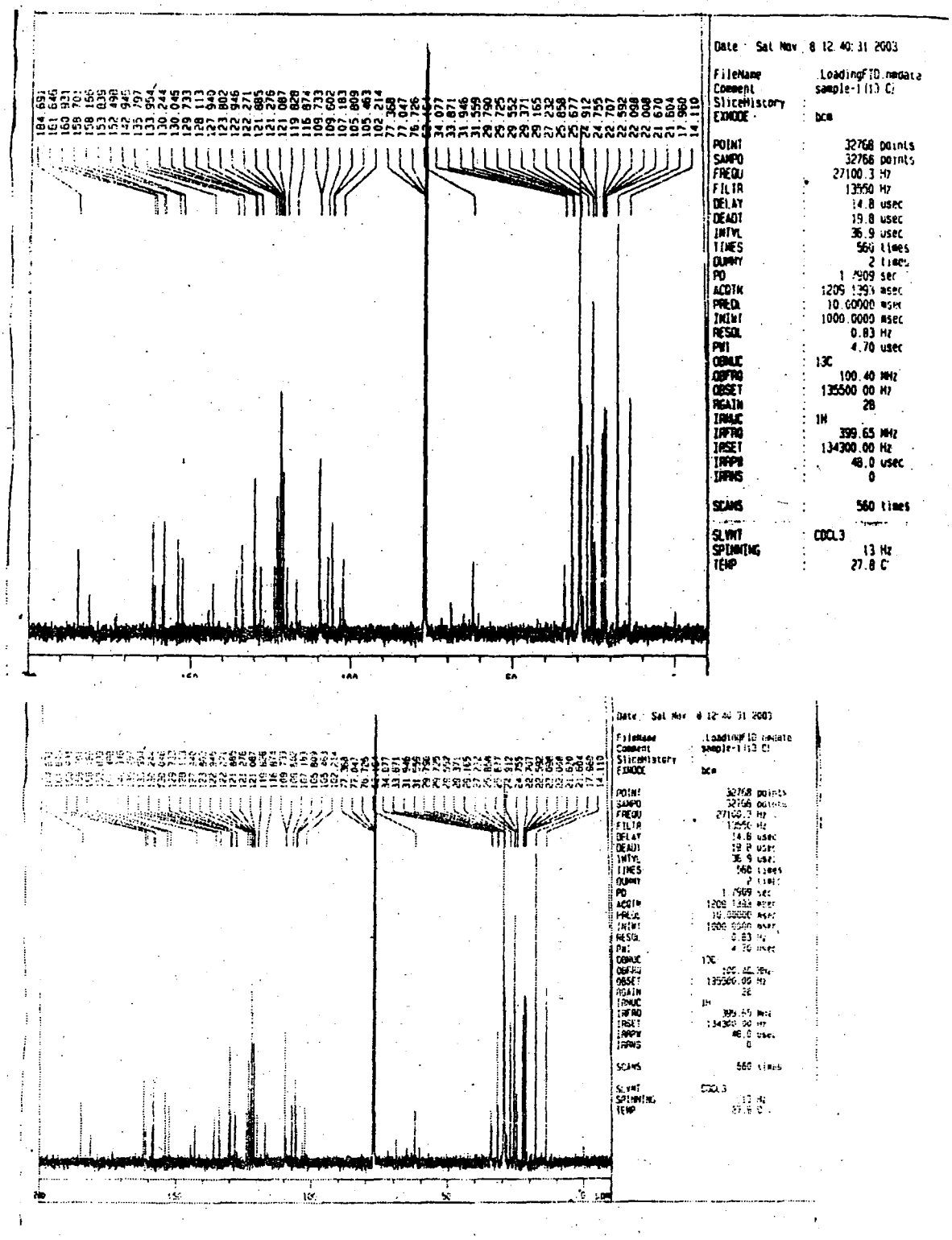

Figure 4. ^{13}C-NMR Spectra of isolated compounds (I and II)

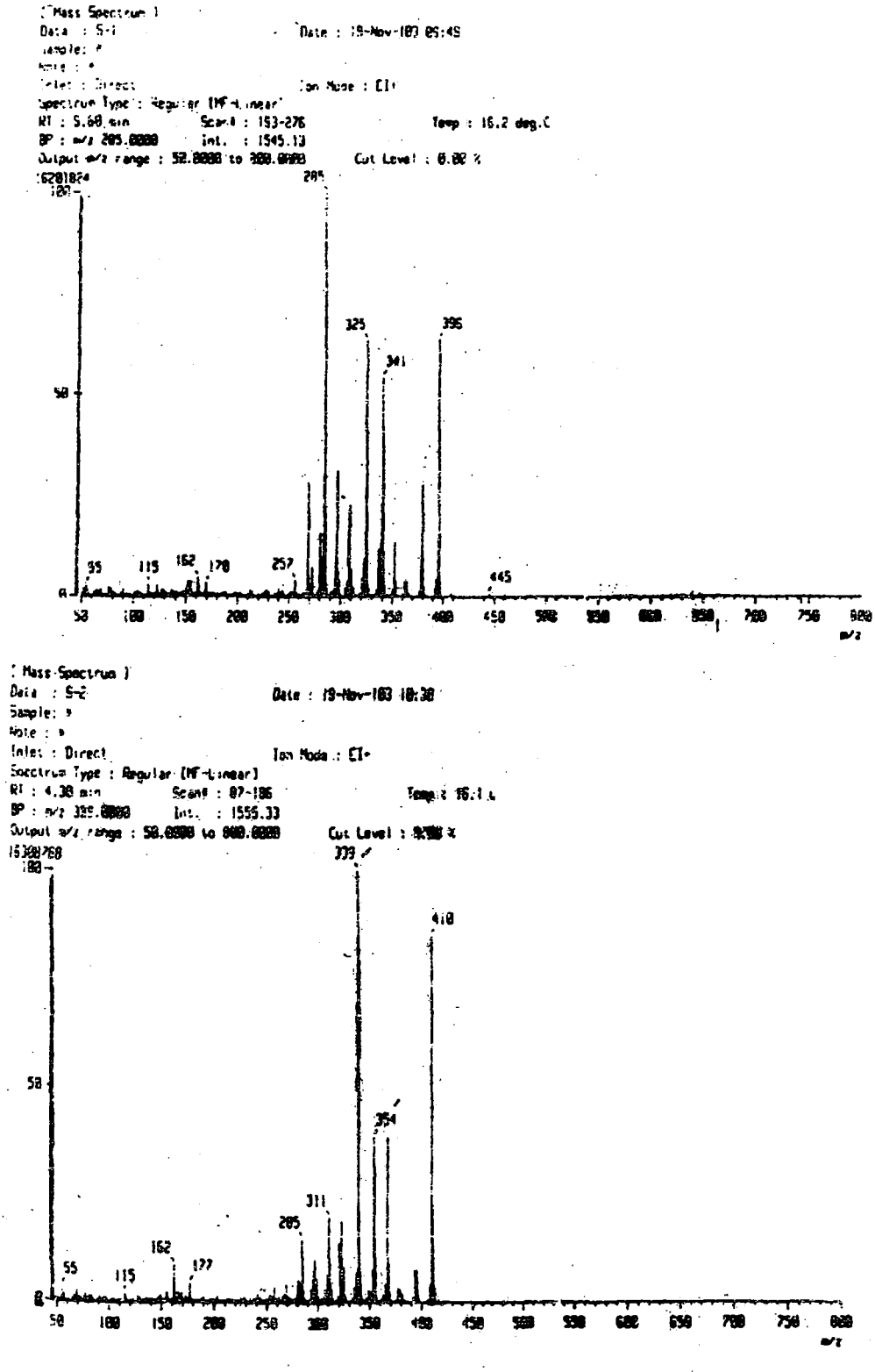

Figure 5. EI Spectra of isolated compounds (I and II)