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Novel anti-inflammatory compound from *Prosopisafricana*

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Abstract. An active natural flavanone compound: 3,7,3,4-tetrahydroxyflavanone, 3-protocatechuate obtained from the stembarks of Prosopis Africana, tested against inflammation and showed a significant anti-inflammatory activity inhibiting COX-2 and iNOS at concentration of 20mM. The structure was established on the basis of their UV, NMR (1D and 2D) spectra, mass spectrometry and polarimeter.

***Key Words*:** Prosopisafricana, anti-inflammatory, flavanone, NMR

Introduction

This Leguminosae family is the most important in the Dicotyledonous group as they contain chemically diverse compounds such as: alkaloids, terpenoids, flavonoids and glycosides which are of interest for their biological activities [1][1].Prosopisafricana plant is found as naturally growing or domesticated in North and the middle of region of Nigeria[2].As part of our ongoing screening of plants against inflammation, Therefore, searching for inhibitory natural therapeutic agents against inflammatory mediators such as Cyclo-oxygenase (COX-2), NOS (iNOS) and prostaglandin E2 could be potential drugs in the treatment of inflammatory diseases[3].

Materials and Methods

General

The chemical profiling of plant extracts was carried out by using pre-coated silica gel plates (0.063-0.020mm, Kieselgel 60 $PF_{254,}$ Merck No. 5554). A range of chromatographic methods were used; such as Column Chromatography (CC) using silica gel (Kieselgel 60; 0.063-0.020mm, Merck), Sephadex LH-20 (Sigma-Aldrich, UK), Vacuum Liquid Chromatography (VLC) using silica gel

(Kieselgel 60H $PF_{254,}$ VWR International Ltd, UK) and Flash chromatography [Flash Master Personal]^{TM}. NMR spectroscopy was extensively used to elucidate the structures of isolated compounds. The IR spectra of samples were recorded in an automatic IR spectrophotometer in the solid state as pressed potassium bromide (KBr) discs. The HRESIMS of all isolated compounds was performed in FTMS-Orbitrap (ThermoFinnigan Bremen, Germany) to give the exact mass of the molecular ion that is useful to determine the molecular formula. Specific rotation of compounds with optical activity was measured by a Perkin Elmer model- 241 polarimeter and an automatic polarimeter Auto pol^{R} V.

Plant material

The stem barks of Prosopisafricanawas collected by Miss HafsatShittu from Albida in Negeria during September 2002. Herbarium specimen was deposited at the phytochemistry Laboratory at the University of Strathclyde, identified at Edinburgh botanical garden and given the voucher number NIPRD/H/6385.

Extraction and isolation

The ground plant materials (AN, PS, PA) were extracted in a Soxhlet apparatus by using different solvent systems starting from non-polar *n*-hexane (60-80°C), semi polar chloroform or ethyl acetate and finally polar solvent as methanol for 2 to 3 days. The extracts were concentrated using a rotary evaporator (BUCHI Labortechnik AG, Switzerland) under reduced pressure at a maximum temperature of 50°C and stored at -20°C before use.

Results and discussion

The compound 1 isolated from the ethyl acetate extract of *Prosopisafricana* as yellowish-brown solid colour, $[\alpha]_D^{21}$ +20 (MeOH, c = 1),R_f=0.29 [MeOH/$CHCl_3$ (1.5:8.5)], TLC analysis showed activity under short-wave light and purple colour spots on spraying with anisaldehyde-sulphuric acid reagent a fter heat, $[M]^+$ at *m/z* 425.0122 corresponding to the molecular formula $C_{22}H_{16}O_9$. I.R (KBr) absorption at 1106, 3420, 1677cm^{-1} were indicative of the presence of –C-O, -OH and –C=O stretching respectively. The experimental UV spectral data showed an absorption bands at λmax^{MeOH} nm= 278.0

The ^{1}H NMR spectrum [400MHz, C_5D_5N, Table 1] showed doublets at δ 8.15 (*J*= 8.4Hz), doublet of doublets at δ 6.88 (*J*= 2.2Hz, 8.8Hz) and 6.77 (*J*= 2.2Hz), were clearly attributed to the protons of C-5, C-6 and C-8 characteristic of a 1,2,4-trisubstituted benzene ring of the A-ring of the flavanone and three broad singlets at δ7.73 (1H, brs) δ7.30 (2H, brs), were assigned as the protons of C-2', C-5'

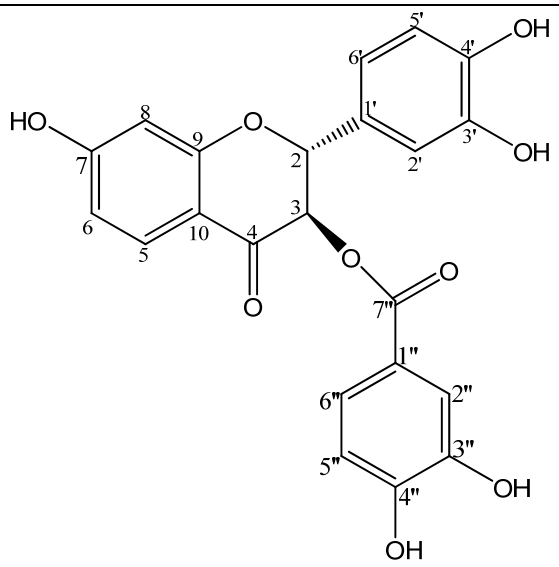
and C6'and characteristic of a 1,3,4-trisubstituted benzene ring (the B-ring of a flavanone). The doublets at δ 5.04 (*J*=11.4Hz) and 5.46 (*J*= 11.4Hz) were assigned as protons of C-3 and C-2, respectively. The coupling constant suggests these protons were in a *trans* relationship. The other doublets at δ 8.38 (*J*= 1.8Hz) and 7.34 (*J*= 8.0Hz) and one doublet of doublets at δ 8.10 (*J*= 8.4Hz, 1.8Hz) due to the protons of C-2'', C-5''and C-6'' respectively characteristic of a protocatechuate type structure. The spin system was confirmed from the COSY spectrum (400MHz, C_5D_5N). Due to the low amount of the sample, the chemical shifts for the carbons were extracted from the HMBC spectrum and the ^{13}C NMR values (Table 1) ranged from δ 74.3 – 194.3 indicated the presence of 22 carbons. The value at δ 194.3 and 169.8 were assigned as ketone and ester carbonyl groups, respectively.

This was confirmed by the HMBC NMR spectrum [Table 1]; long range correlations were observed and confirmed the signals of δ 5.46 and 5.04 of H-2 and H-3 showing correlations to ^2J and ^3J respectively to C-1' of ring- B whereas H-2 showing ^3J correlation to C-9 of ring-A. H-3 showing

^{2}J to carbonyl at C-4 of ring- C and ^{3}J to C-1' and ^{2}J to C-2 and ^{4}J to C-6' and C-2' and H-3 showing ^{3}J correlation to C-7'' of a protocatechuate ester substituent attached to C-3 of ring C.
In the HMBC spectrum, H-8 showed ^{3}J correlation to C-6 and ^{4}J correlation to C-4. Thus, the compound 1 was identified as 3,7,3',4'-tetrahydroxyflavanone, 3-protocatechuate. The compound 1is a novel natural product and has not been found in any literature search (DNP, version 16:2 and Chemical Abstract).

Table 1: ^{1}H (400MHz), ^{13}C and HMBC NMR data of compound 1 in C_5D_5N

Position	^{1}H	^{13}C	HMBC (H →C)
2	5.46, d, *J*= 11.4Hz	85.5	C-3, C-4, C-9, C- 1', C-2', C-6'
3	5.04, d, *J*= 11.4Hz	74.3	C-2, C-4, C-1'
4		194.3	
5	8.15 d, *J*= 8.4Hz	129.7	C-4, C-7, C-9
6	6.88, dd, *J*= 2.2Hz, 8.4Hz	112.0	C-8, C-10
7		167.1	
8	6.77, d, *J*= 2.2Hz	103.6	C-7, C-9, C-10
9		164.6	
10		113.7	
1'		130.2	
2'	7.73, brs	116.7	C-1', C-2, C-3', C-4', C-6'
3'		146.9	
4'		148.9	
5'	7.30, brs	116.4	C-2, C-1', C-2', C-4'
6'	7.30, brs	120.5	C-2, C-1', C-2', C-4'
7''		169.8	
1''		124.2	
2''	8.38, d, *J*= 1.8Hz	118.2	C-7'', C-3'', C-4'', C-6''
3''		147.4	
4''		152.5	
5''	7.34, d, *J*= 8.0Hz	116.2	C-1'', C-3'', C-4''
6''	8.10, dd, *J*= 8.4Hz, 1.8Hz	123.3	C-7'', C-2'', C-3'', 4''



1

Effect on the induction of iNOS and COX-2

The Figures 1, 2, 3 and 4 showed the effect of compound **1** on the COX-2 and iNOS proteins at a three different concentrations1 (100µM), concentration 2 (10µM) and concentration 3 (1µM) as obtained by western blot analysis.

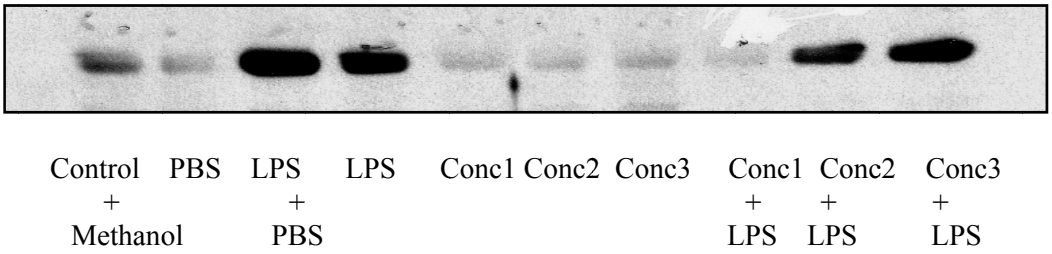


Figure 1: Western blot analysis of LPS dependent COX –2 protein expression for Compound **1** in RAW264.7 cells
(PBS): Phosphate buffer saline, (LPS): Lipopolysaccharide

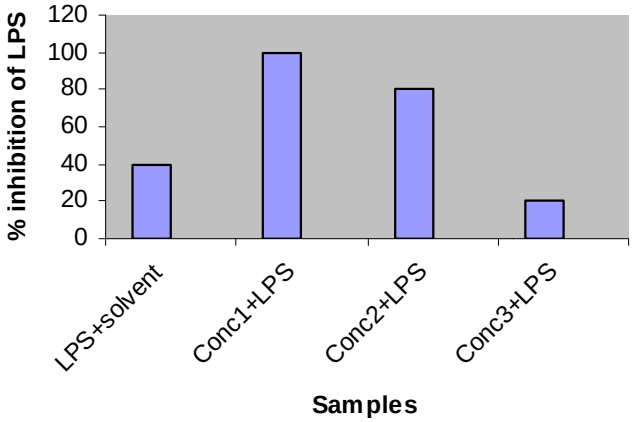


Figure 2: % inhibition of the LPS dependent COX- 2 protein expression for compound **1** in RAW 264.7

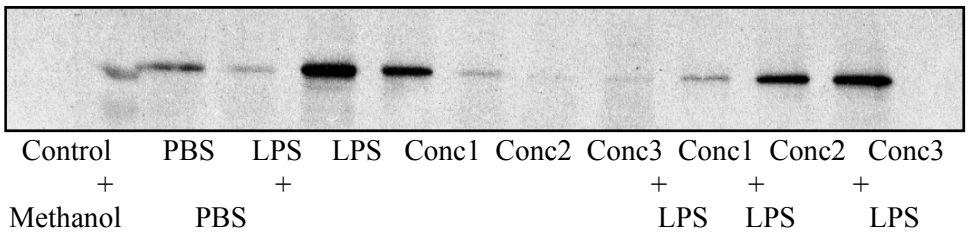


Figure 3: Western blot analysis of LPS dependent iNOS protein expression for compound **1** in RAW264.7 cell

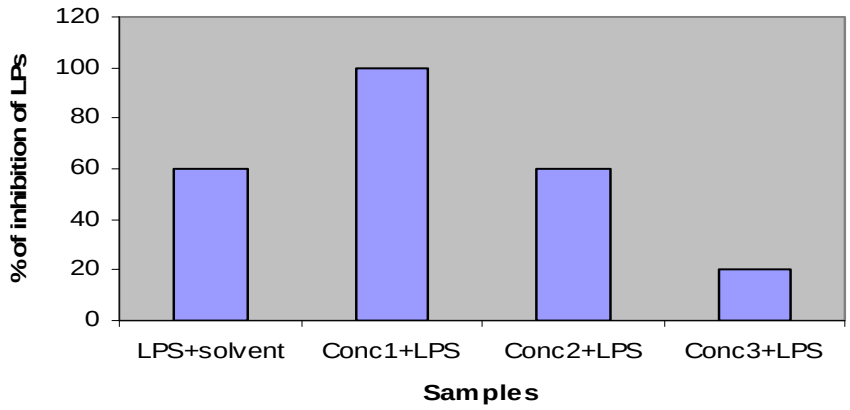


Figure 4: % inhibition of the LPS dependent iNOS protein expression for compound **1** in RAW 264.7 Samples; LPS+solvent (1), Conc1+LPS (2), Conc2+LPS (3) and Conc3+LPS (4)

Compound **1** showed significant inhibition on iNOS (Inducible Nitric Oxide Synthase) and COX-2 protein in RAW 264.7 cells following from LPS (Lipopolysaccharide) stimulation. Different concentrations of JE13 ranging from 1, 10 and 100 µlM with LPS showed different inhibitory effects whereas LPS alone showed highest iNOS (Inducible Nitric Oxide Synthase) and COX-2 (Cyclo-oxygenase-2) induction and was used as a positive control whereas methanol as a negative control. The inhibition of NOS in RAW 264.7 cells could be due to 7-OH and 3',4'-hydroxy groups. This effect supports the anti-inflammatory properties of reported literature [4]. Also the other flavonoids such as quercetin, genistein and daidzein with 5,7-hydroxy groups support the present wok of compound 1as mentioned in the previous reports[5].

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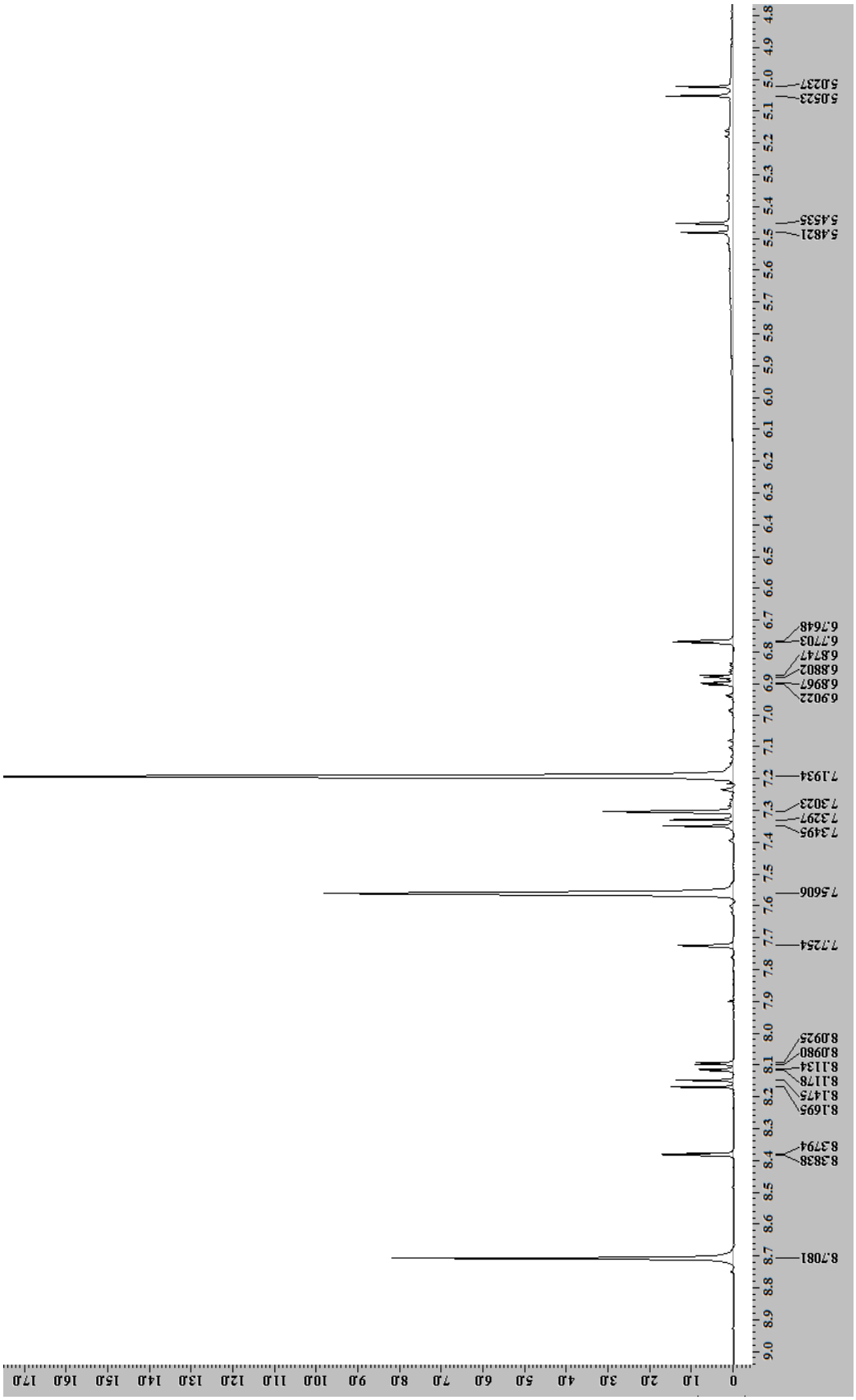
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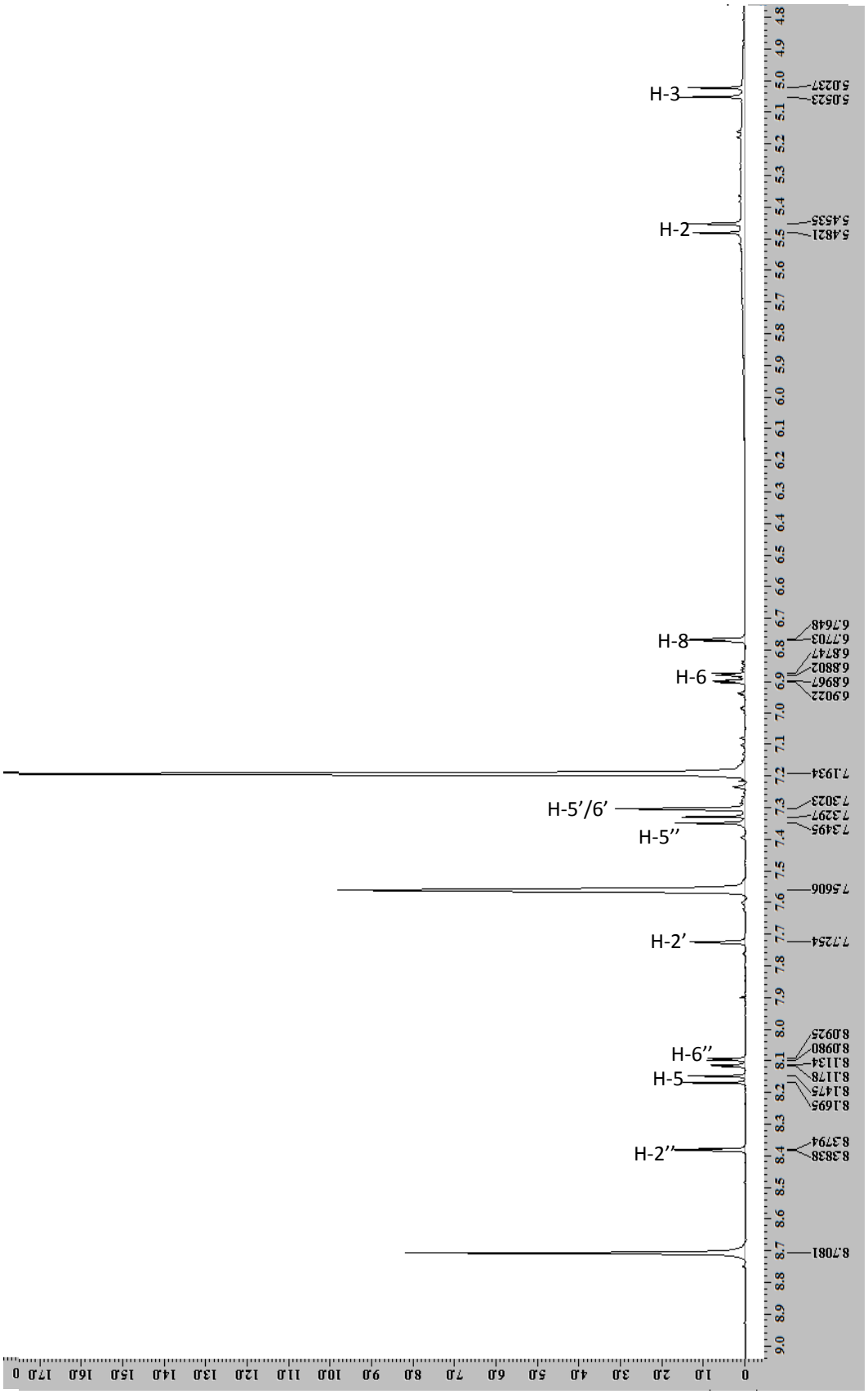
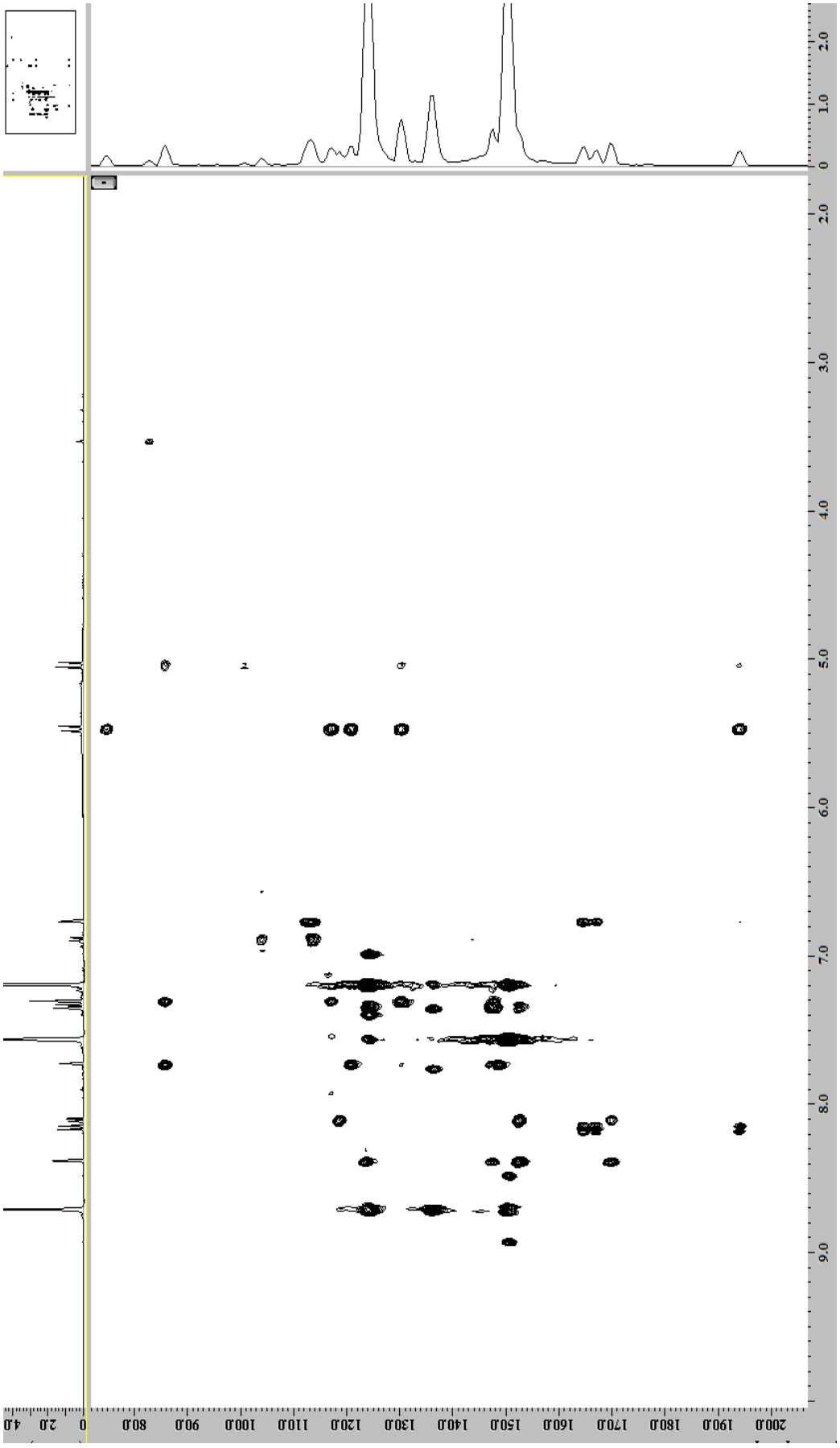
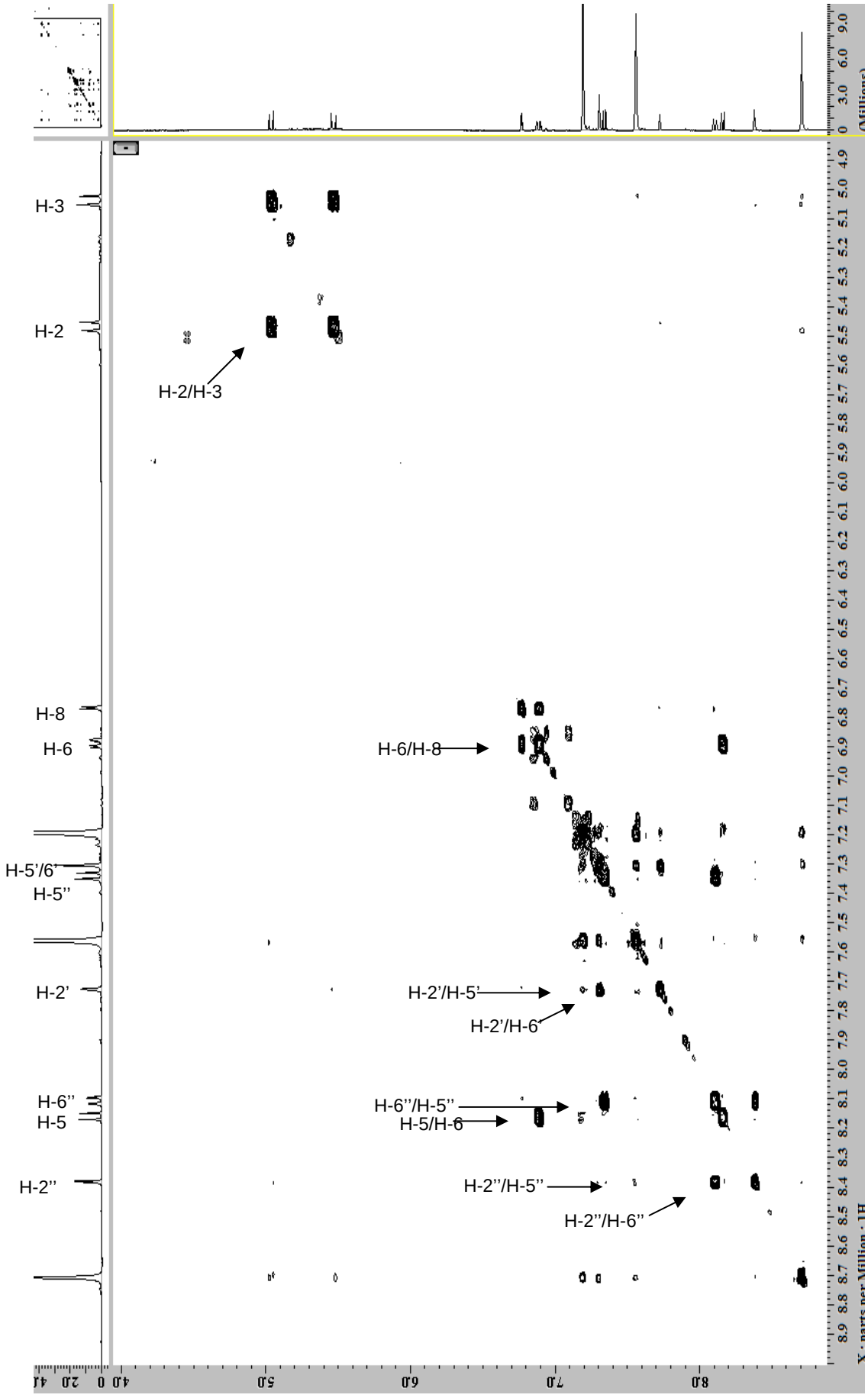


^{1}H NMR spectrum (400MHz, C_5D_5N) of JE13 as 3,7,3',4'-tetrahydroxyflavanone, 3-protocatechuate





^{1}H-^{1}H COSYspectrum (400MHz, C_5D_5N) of JE13 as 3,7,3',4'-tetrahydroxyflavanone, 3-protocatechuate