Supporting Information to

Antiprotozoal and Cytotoxic Studies on some Isocordoin Derivatives

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Experimental Procedures

***Preparation of 2',4'-diacetoxy-3'-(3-methylbut-2-enyl)chalcone* (2)**

A mixture of **1** (8 mg, 0.26 mmol), acetic anhydride (1 mL), and pyridine (2 mL) was allowed to stir overnight at room temperature. The reaction mixture was poured over water (30 mL) and the resulting suspension extracted with ethyl acetate (1:1, 3x). The organic layer was washed (1:1, v:v) successively in water, 5% HCl, 5% $NaHCO_3$, and a NaCl saturated solution. The treatment of the organic layer with anhydrous Na_2SO_4 followed by filtration and evaporation yielded 9.2 mg (0.23 mmol) of pure compound **2**; yield: 80%.

***Preparation of 2',4'-Dimethoxy-3'-(3-methylbut-2-enyl)chalcone* (3) and *2'-hydroxy, 4'-methoxy-3'-(3-methylbut-2-enyl chalcone* (4)**

A mixture of **1** (35 mg, 0.11 mmol), CH_3I (200 µL) and K_2CO_3 (110 mg) in 2 mL of acetone was left to stir overnight at room temperature and then diluted with 10 mL of water. The resulting solution was extracted with ethyl acetate (2x, 20 mL). The crude reaction product presented two major components by TLC. The reaction mixture was purified by prep. TLC (*n*-hexane:acetone, 8:2) to give metabolite **3** (8.1 mg, 0.024 mmol) and **4** (20 mg, 0.066 mmol). This reaction was repeated twice in order to obtain additional amounts of **3** and **4** for biological evaluation.

***Preparation of 2",2"-dimethypyran (5",6"3',4')-2'-hydroxychalcone* (5)**

55 mg of chalcone **1** (0.062 mmol) were heated under reflux for 24 h with 13 mL EtOH, 7 mL H_2O and 1.5 mL conc. HCl. After the reaction mixture was complete (TLC monitoring) the contents were poured into ice-water. The solid obtained was purified on gel permeation column chromatography using sephadex LH20 and eluting with MeOH to yield 23 mg (0.021 mmol) of **5**.

***Preparation of 2",2"-dimethylpyran (5",6":2',3')-4'-methoxychalcone* (6)**

Modification of the prenyl chain of **4** was performed following the same procedure as in **5**: 20 mg of chalcone **4** were heated under reflux for 24 h with 5 mL EtOH, 2.5 mL H_2O and 0.5 mL conc. HCl. After the reaction mixture was complete (TLC monitoring) the contents were poured into

ice-water. The solid obtained was recrystallized from EtOH to give 6.8 mg of compound **6** (34.5%).

Preparation of 2',4'-dihydroxychalcone **(8)**

A solution of benzaldehyde (106 mg, 1 mmol) and 2',4'-dihydroxyacetophenone (152 mg, 1 mmol) and sodium hydroxide in 25 mL of EtOH was stirred at ambient temperatures for 24 h. The crude product obtained was purified in CC (*n*-hexane:EtOAc 9:1), followed by sephadex LH20 in MeOH to obtain 6 mg of pure metabolite 8 (2.5%).

In vitro **antileishmanial assay**

The growth inhibition test was perfomed on promastigotes of *Leishmania mexicana* strain (MHOM/MX/ISETGS). Parasites were cultured at 26 °C in Schneider's drosophila medium (Sigma), supplemented with 10% fetal bovine serum (Gibco), penicillin (100 IU/mL; Sigma), and streptomycin (100 µg/mL; Sigma). Assays were perfomed in 96-well plates and all compounds were evaluated in duplicated. Compounds were solubilized in dimethyl sulfoxide (DMSO; Omnisolv) and diluted with liquid medium. A mixture of 100 µL of compound solution and 100 µL of culture medium containing 10,000 parasites was added to obtain concentrations of 100, 50, 25, and 12.5 µg/mL. Pentamidine (Sigma) was used as a positive control and a control containing parasites without compound solution was also included. The plate was incubated at 26 °C for 72 h and the leishmanicidal activity of compounds was determined by direct count of parasites in a Neubauer chamber under a light microscope. The IC_{50} of each compound was calculated by a Probit analysis.

In vitro **trypanocidal assay**

The inhibition of epimastigotes growth was assessed by a modification of the method described by Hocquemiller et al. *T. cruzi* epimastigotes (MHOM/MX/1994/INC5) were cultured at 26 °C in Schneider's drosophila medium (Sigma), supplemented with 10% fetal bovine serum (Gibco), penicillin (100 IU/mL; Sigma) and streptomycin (100 µL/mL; Sigma). Assays were perfomed in 96-well plates and all compounds were evaluated in duplicate. Compounds were solubilized in dimethyl sulfoxide (DMSO; Omnisolv) and diluted with liquid medium. A mixture of 100 µL of compound solution and 100 µL of culture medium containing 20,000 parasites was added to

obtain concentrations of 100, 50, 25 and 12.5 μg/mL. Nifurtimox and benznidazole were used as positive controls and a control containing parasites without compound solution was also included. The plate was incubated at 26 °C for 72 h and the trypanocidal activity of compounds was determined by direct count of parasites in a Neubauer chamber under a light microscope. The IC_{50} of each compound was calculated by a Probit analysis.

Cytotoxicity test

The cytotoxicity assay was performed according to Rahman et al. where 1.5 x 10^4 viable cells from the cell line were seeded in a 96-well plate (Costar) and incubated for 24 to 48 h [16]. Dog kidney cells (MDCK) were grown in DMEM (Gibco) media supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco) with 100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin and maintained at 37 ^oC in a 5% CO^2 atmosphere with 95% humidity. When cells reached >80% confluence, the medium was replaced and the cells were treated with the compounds at 6.25, 12.5, 25, and 50 μg/mL dissolved in dimethyl sulfoxide (DMSO) at a maximun concentration of 0.05%. After 72 h of incubation, 10 μL of a 0.005% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma) solution (5 mg/mL) was added to each well and incubated at 37 ^{o} C for 4 h. The medium was removed and the formazan, a product generated by the activity of dehydrogenases in cells, was dissolved in acidified isopropanol (0.4 N HCl). The amount of MTT-formazan is directly proportional to the number of living cells and was determined by measuring the optical density (OD) at 540 nm using a bioassay reader (BioRad). Docetaxel was used as a positive control whereas untreated cells were used as negative control. The concentration of the compound that killed 50% of the cells (CC_{50}) was calculated by GraphPad Prim 4 software. All determinations were performed in triplicate. The selectivity index (SI) of the compounds was defined as the ratio of cytotoxicity to biological activity, as calculated.

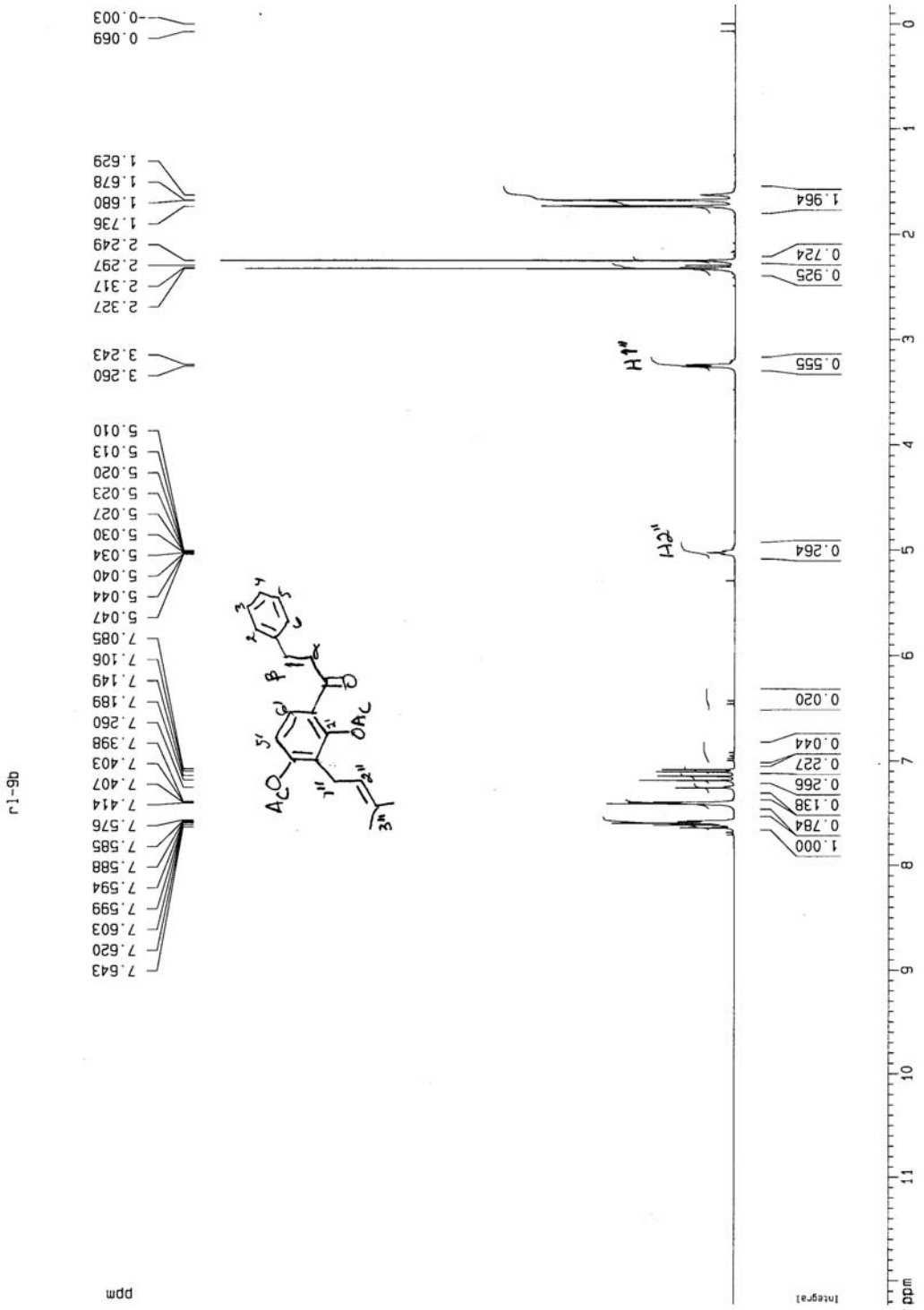

Fig. 1S The [1]H-NMR spectrum (400 MHz) of **2**.

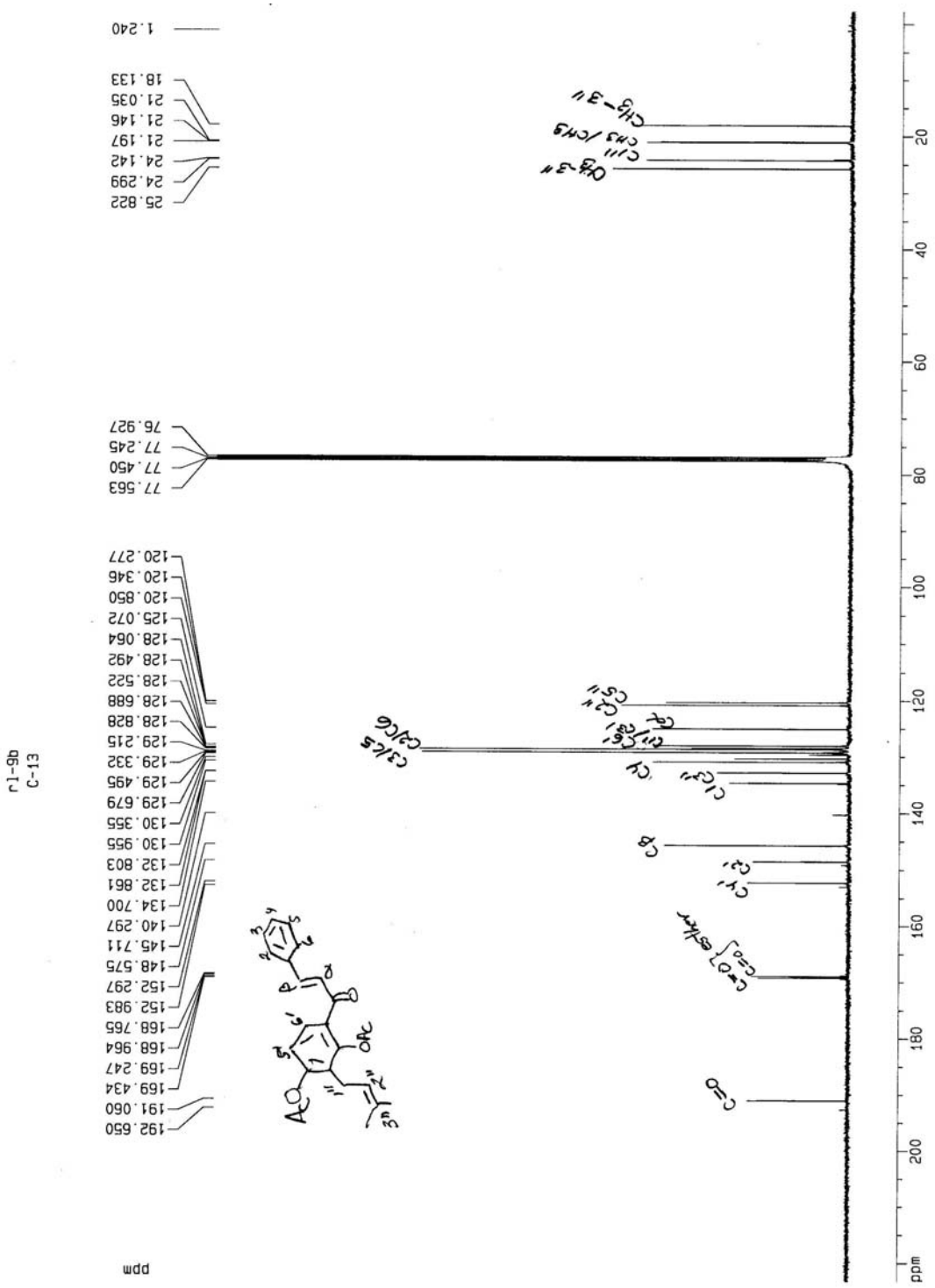

Fig. 2S The ^{13}C-NMR spectrum (100 MHz) of **2**.

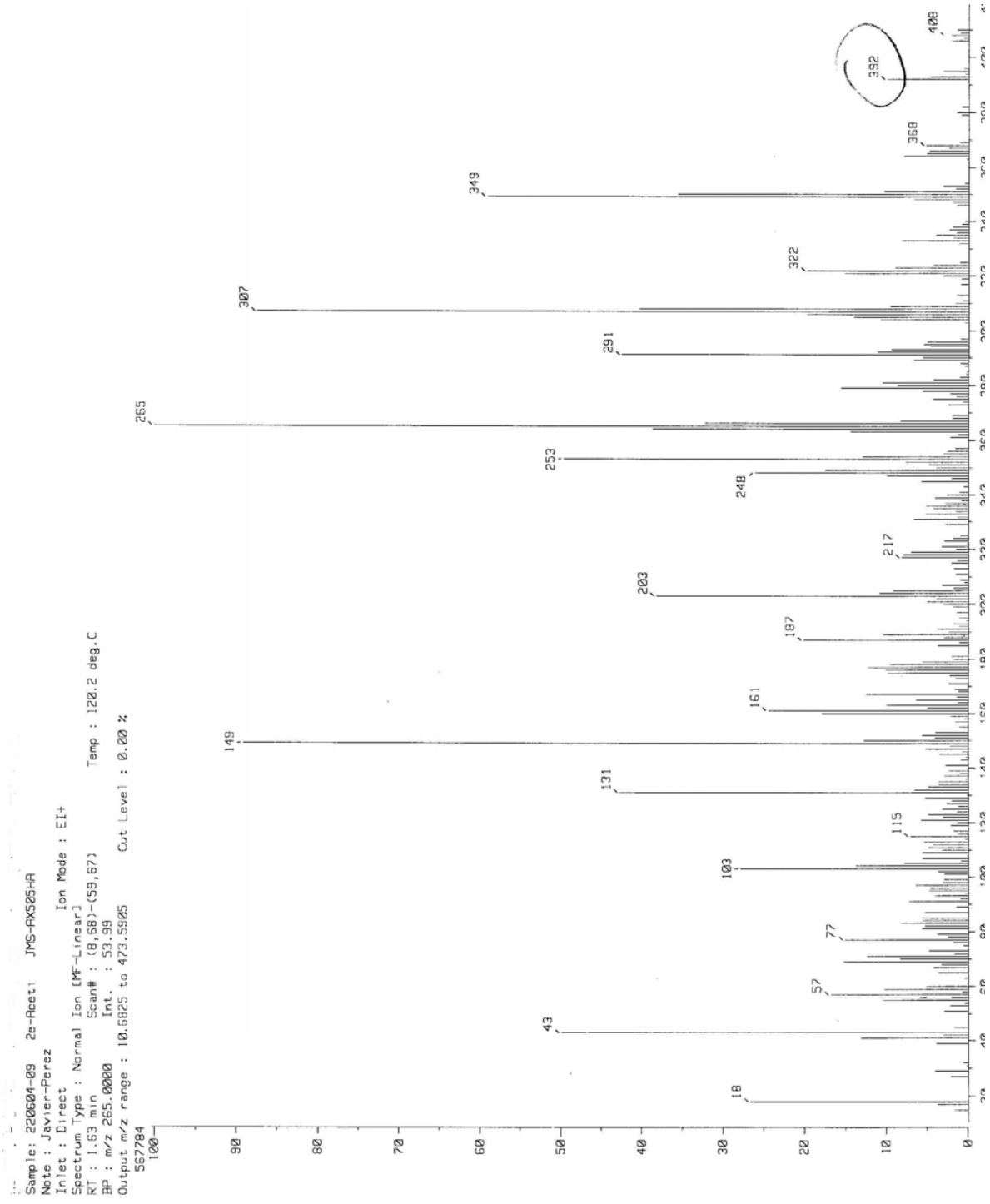

Fig. 3S The mass spectra of **2**.

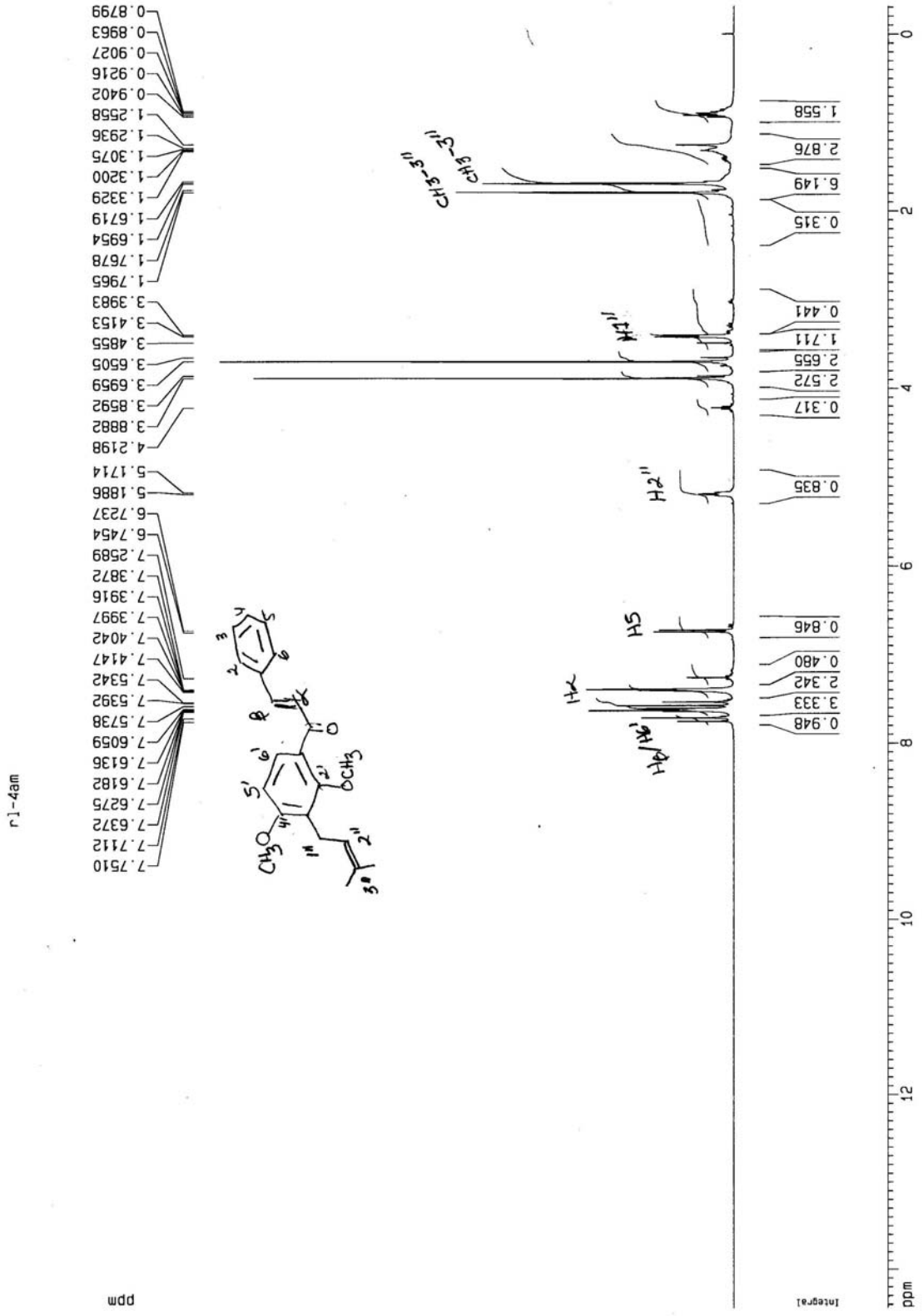

Fig. 4S The [1]H-NMR spectrum (400 MHz) of **3**.

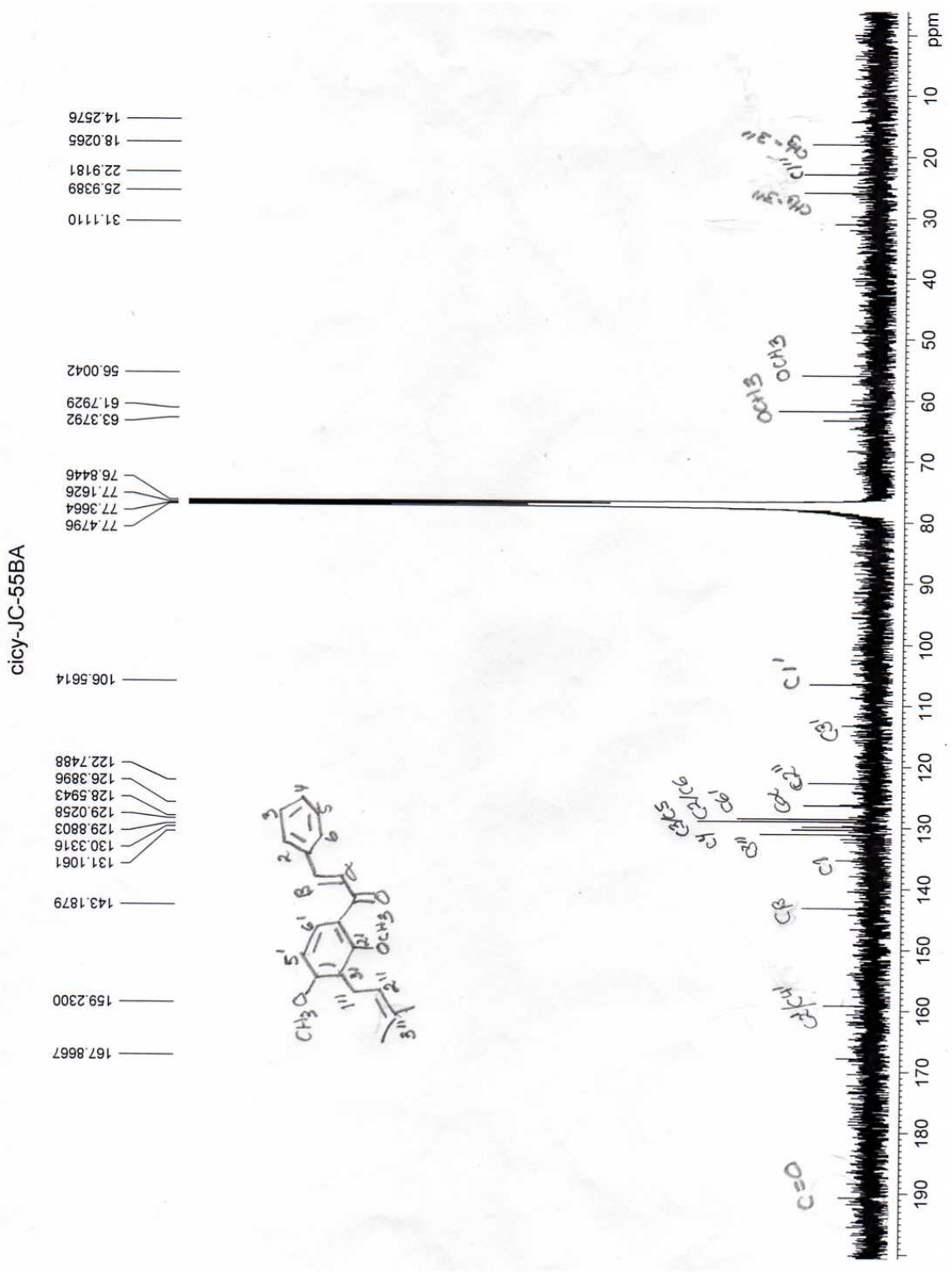

Fig. 5S The ^{13}C-NMR spectrum (100 MHz) of **3**.

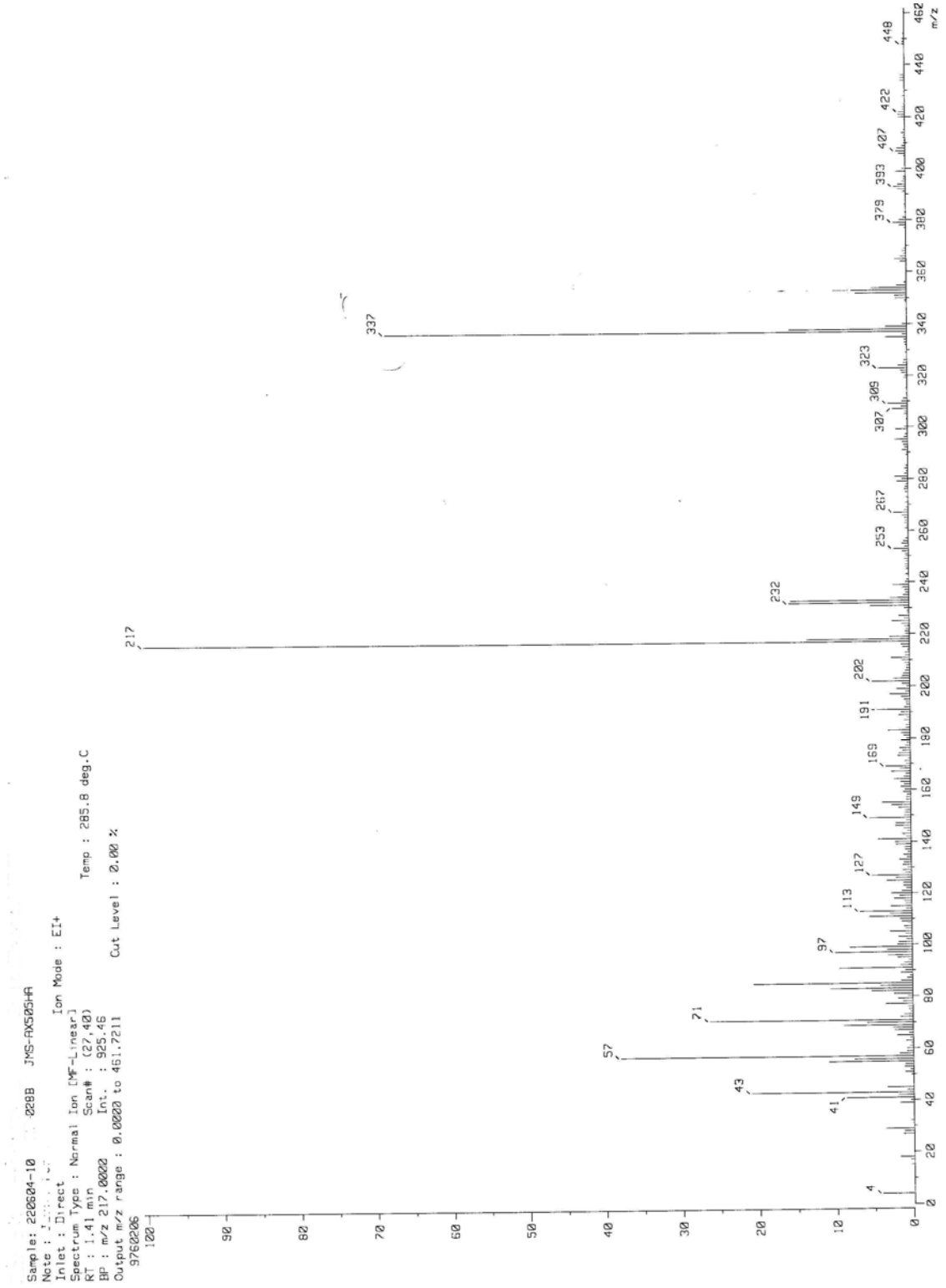

Fig. 6S The mass spectra of **3**.