

# Synthesis of Pelorol and Analogues: Activators of the Inositol 5-Phosphatase SHIP

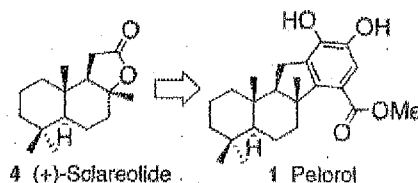
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## ABSTRACT



A screening program designed to find new antiinflammatory agents has identified the sponge meroterpenoid pelorol (1) as an in vitro activator of the inositol-5-phosphatase SHIP. Pelorol (1) and several functional group analogues have been synthesized from sclareolide (4).

The phosphatidylinositol-3-kinase (PI3K) signaling pathway plays an important role in the regulation of many cellular functions, including survival, adhesion, movement, proliferation, differentiation, and end cell activation.<sup>1</sup> A key second messenger in this pathway is the membrane-associated phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P<sub>3</sub>), which is present in low levels in unstimulated cells but is rapidly synthesized from PI-4,5-P<sub>2</sub> by PI3K in response to a diverse set of extracellular stimuli (Figure 1). To ensure that activation of the PI3K pathway is appropriately restrained, the tumor suppressor PTEN hydrolyzes PI-3,4,5-P<sub>3</sub> back to PI-4,5-P<sub>2</sub> and the Src homology 2-containing inositol 5-phosphatases SHIP, sSHIP, and SHIP2 hydrolyze it to PI-3,4-P<sub>2</sub>.

Approximately 50% of human cancers contain biallelic inactivating mutations of the ubiquitously expressed PTEN, which illustrates the importance of these phosphatases in preventing uncontrolled cell growth. Similar to PTEN, SHIP2 is expressed in a wide variety of cell types, whereas sSHIP is restricted to stem cells and SHIP is found only in hematopoietic (blood) cells.

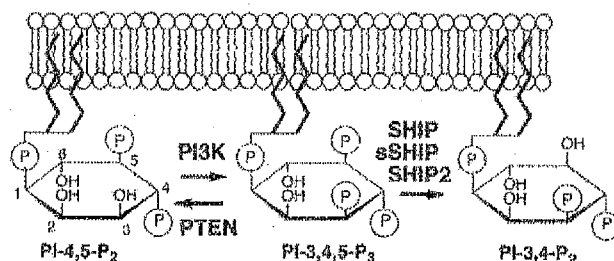


Figure 1. Enzymatic synthesis and degradation of PI-3,4,5-P<sub>3</sub>.

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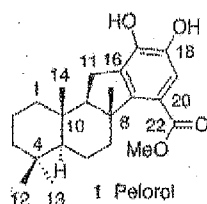
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(1) (a) Kalesnikoff, J.; Sly, L. M.; Hughes, M. R.; Buchse, T.; Rauh, M. J.; Cao, L.-P.; Lam, V.; Mui, A.; Huber, M.; Krystal, G. *Rev. Physiol. Biochem. Pharmacol.* **2003**, *149*, 87-103. (b) Rauh, M. J.; Kalesnikoff, M.; Hughes, M.; Sly, L.; Lam, V.; Krystal, G. *Biochem. Soc. Trans.* **2003**, *31*, 286-291. (c) Rauh, M. J.; Krystal, G. *Clin. Invest. Med.* **2002**, *25*, 68-69. (d) Krystal, G. *Semin. Immunol.* **2002**, *12*, 397-403.

Krystal and co-workers have generated mice containing a homozygous deletion of SHIP (SHIP<sup>-/-</sup> mice).<sup>2</sup> These animals are viable and fertile but typically do not survive beyond 14 weeks because of a myeloproliferative disorder. Experiments with SHIP<sup>-/-</sup> mice and with SHIP<sup>-/-</sup> bone-marrow-derived mast cells (BMMC) and macrophages (BMMφs) obtained from these mice, have demonstrated that SHIP is a negative regulator of immunoglobulin E (IgE) or Steel Factor induced mast cell activation,<sup>3</sup> a negative regulator of lipopolysaccharide (LPS) induced macrophage activation, and a negative regulator of osteoclast formation and resorptive function. As a result of this last property, SHIP<sup>-/-</sup> mice suffer from severe osteoporosis.<sup>4</sup> There is also evidence that SHIP acts as a tumor suppressor in both acute myelogenous leukemia (AML)<sup>5</sup> and in chronic myelogenous leukemia (CML).<sup>6</sup>

Current attempts to develop drugs based on intervention in signaling pathways are overwhelmingly biased toward finding selective kinase inhibitors. There has been some recent interest in examining the therapeutic potential of phosphatase inhibitors,<sup>7</sup> but there has been virtually no effort to explore the usefulness of small-molecule phosphatase activators. The important role of SHIP as a negative regulator of mast cell and macrophage activation, osteoclast formation, and resorptive function, as well as in AML and CML, combined with its occurrence only in hematopoietic cells, makes it an attractive drug target. We hypothesized that selective activators of SHIP would be useful experimental tools and potential drug candidates to provide proof of principle validation for a new approach to the treatment of inflammation, osteoporosis, and leukemia.

Crude extracts of marine invertebrates were screened for in vitro activation of the SHIP-catalyzed conversion of inositol-1,3,4,5-tetrakisphosphate (IP<sub>4</sub>) to inositol-1,3,4-trisphosphate (IP<sub>3</sub>).<sup>8</sup> A MeOH extract of the sponge *Dactylospongia elegans* (Thiele, 1899), collected in Papua New Guinea,<sup>9</sup> showed promising activity in the assay. Bioassay-guided fractionation of the extract identified pelorol (**1**) as the sole SHIP-activating component. Three related mero-



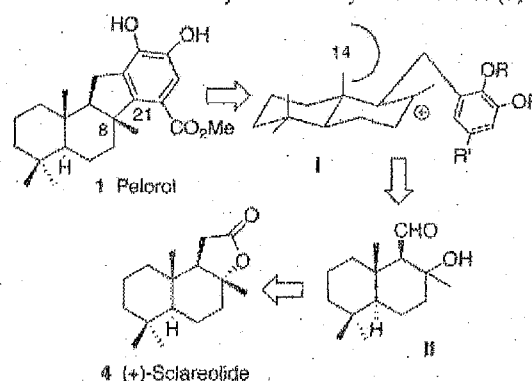
terpenoids, ilimaquinone,<sup>10</sup> mamanuthaquinone,<sup>11</sup> and dactyloquinone A,<sup>12</sup> were also isolated from the *D. elegans* extract but were not active in the assay. Pelorol (**1**) was

undescribed when first isolated in our laboratory as a SHIP activator, but while further biological studies were in progress it was isolated by König's group, also from *D. elegans*,<sup>13</sup> and by Schmitz's group from *Petrospongia metachromia*.<sup>10</sup> Spectroscopic data obtained for pelorol in the current work was in complete agreement with the data reported by König and Schmitz.

The limited quantity (~10 mg) of pelorol (**1**) available from the source sponge *D. elegans* was inadequate to support detailed in vitro and in vivo evaluation of its ability to activate SHIP. To satisfy the need for additional material, confirm the absolute configuration of the natural product, and generate analogues for SAR, the total synthesis of pelorol (**1**) and analogues where the methyl ester at C-20 was replaced by methyl and ethyl residues was undertaken.

On the basis of sound biogenetic arguments, Schmitz predicted that the absolute configuration of pelorol (**1**) was 5*S*,8*R*,9*R*,10*S* as drawn. Therefore, the starting material selected for the synthesis of pelorol and analogues was the commercially available terpenoid (+)-sclareolide (**4**), which has the same absolute configurations at C-5, C-9, and C-10 as those predicted for pelorol (**1**). The synthetic plan anticipated that the key reaction would involve a biomimetic carbocation-initiated cyclization of an intermediate **I** to generate the C-8/C-21 bond (Scheme 1). Steric bulk associ-

Scheme 1. Retrosynthetic Analysis of Pelorol (**1**)



ated with the C-14 methyl was expected to cause preferential approach of the phenyl ring from the bottom face of C-8 to form the required trans B/C ring fusion. Synthetic routes to both C-8 epimers (**5** and **15**) of **II** starting from sclareolide have been reported, and the plan was to examine both as

(2) Helgason, C. D.; Damen, J. E.; Rosten, P.; Grewal, R.; Sorensen, P.; Chappel, S. M.; Borowski, A.; Jirik, F.; Krystal, G.; Humphries, R. K. *Genes Dev.* 1998, 12, 1610-1620.

(3) Huber, M.; Helgason, C. D.; Damen, J. E.; Liu, L.; Humphries, R. K.; Krystal, G. *Proc. Natl. Acad. Sci. U.S.A.* 1998, 95, 11330-11335.

(4) Takashita, S.; Namba, N.; Zhuo, J. J.; Jiang, Y.; Genant, H. K.; Silva, M. J.; Brodt, M. D.; Helgason, C. D.; Kalesnikoff, J.; Rauh, M. J.; Humphries, R. K.; Krystal, G.; Teitelbaum, S. L.; Ross, F. P. *Nat. Med.* 2002, 8, 943-949.

(5) Luo, J.-M.; Yoshida, H.; Komura, S.; Ohishi, N.; Pan, L.; Shigeno, K.; Hanamura, I.; Miura, K.; Iida, S.; Ueda, R.; Naoki, T.; Akao, Y.; Ohno, R.; Ohnishi, K. *Leukemia* 2003, 17, 1-8.

(6) Sattler, M.; Salgia, R.; Shrikhande, G.; Verma, S.; Choi, J.-L.; Rohrschneider, L. R.; Griffin, J. D. *Oncogene* 1997, 15, 2379-2384.

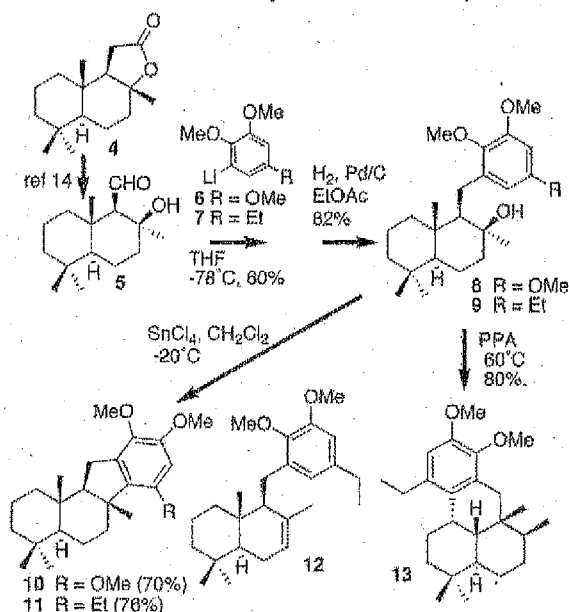
(7) McCluskey, A.; Sim, A. T. R.; Sakoff, J. A. *J. Med. Chem.* 2002, 45, 1151-1175.

(8) The SHIP assay was performed in 96-well microtitre plates with 10 ng of recombinant SHIP enzyme per well. SHIP enzyme was incubated with extract or DMSO for 15 min at 23 °C before addition of 200 mM inositol-1,3,4,5-tetrakisphosphate. The reaction was allowed to proceed for 20 min at 37 °C and the amount of inorganic phosphate released was then assessed by the addition of Malachite Green reagent followed by an absorbance measurement at 650 nm.

intermediates in the preparation of direct precursors to the carbocation **1** and ultimately cyclized products.

(+)-Sclareolide (**4**) was converted to the aldehyde **5** following the literature procedure (Scheme 2).<sup>14</sup> Overman

Scheme 2. Initial Attempts at Biomimetic Cyclizations



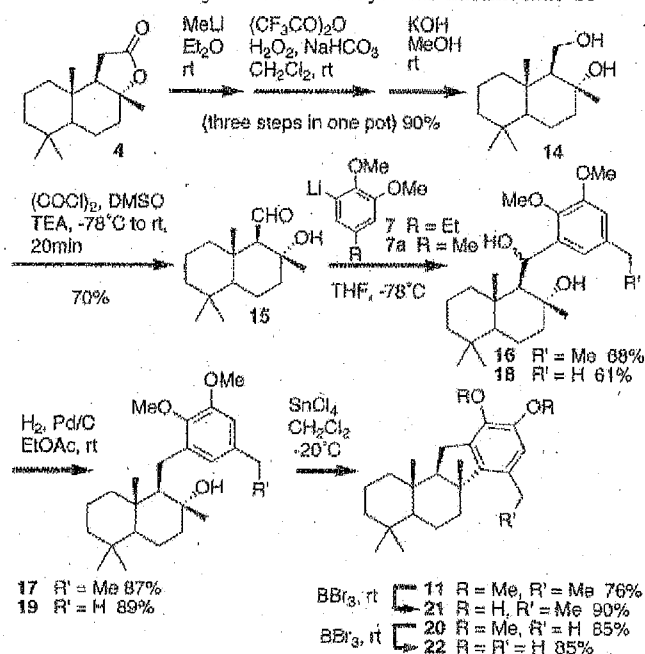
showed in his synthesis of adociasulfate that a highly nucleophilic arene was required to trap a carbocation such as **1** in preference to proton elimination to give an uncyclized olefin.<sup>15</sup> Therefore, trimethoxyphenyllithium **6** (2 equiv) was added to aldehyde **5**, followed by hydrogenolysis of the resulting epimeric benzyl alcohols, to give the tertiary alcohol **8**. Treatment of **8** with  $\text{SnCl}_4$  gave the cyclization product **10** having the desired stereochemistry in good yield. Unfortunately, all attempts to transform 1,2,4-trialkoxy benzene **10** or various alkyl ether analogues into polorol failed.

In an attempt to overcome this problem, the coupling reaction was repeated with dimethoxyethylphenyllithium **7** to give the tertiary alcohol **9**. Initial reactions of **9** with  $\text{SnCl}_4$  gave variable yields of the desired product **11** and the undesired elimination product **12**, while treatment with the protic acid PPA gave only the unanticipated cyclization product **13**. We envisaged that the formation of **12** and **13** resulted from reduction of the nucleophilicity of the arene

( $\text{R} = \text{Et}$  vs  $\text{R} = \text{OMe}$ ) making the elimination reaction leading to **12** and the Wagner Meerwein rearrangements leading to **13** competitive with direct trapping of the C-8 carbocation by the arene to give the desired product **11**. After optimization of the reaction conditions, it was found that the  $\text{SnCl}_4$ -catalyzed cyclization gave consistently high yields ( $\sim 76\%$ ) for the conversion of **9** to **11**.

Although it was possible to obtain **11** via the aldehyde **5**, the preparation of **5** from sclareolide was cumbersome because it required multiple chromatographic separations and preparation of an oxidizing agent that was not commercially available.<sup>14</sup> Therefore, we turned our attention to the aldehyde **15**, having an equatorial OH group at C-8 (Scheme 3).<sup>16</sup> (+)-

Scheme 3. Synthesis of the Cyclized Intermediate **11**



(9) A voucher sample has been deposited at the Zoological Museum, University of Amsterdam (ZMA POR. 15986).

(10) Kwak, J. H.; Schnitz, P. J.; Kelly, M. *J. Nat. Prod.* **2000**, *63*, 1153–1156.

(11) Swersey, J. C.; Barrows, L. R.; Ireland, C. M. *Tetrahedron Lett.* **1991**, *32*, 6687–6690.

(12) Mitome, H.; Nagasawa, T.; Miyaoka, H.; Yamada, Y.; van Soest, R. W. M. *J. Nat. Prod.* **2001**, *64*, 1506–1508.

(13) Goclik, E.; König, G. M.; Wright, A. D.; Kaminsky, R. *J. Nat. Prod.* **2000**, *63*, 1150–1152.

(14) Quidéau, S.; Lebon, M.; Lamidey, A.-M. *Org. Lett.* **2002**, *4*, 3975–3978.

(15) Bogenstaetter, M.; Limberg, A.; Overman, L. E.; Tomasi, A. L. *J. Am. Chem. Soc.* **1999**, *121*, 12206–12207.

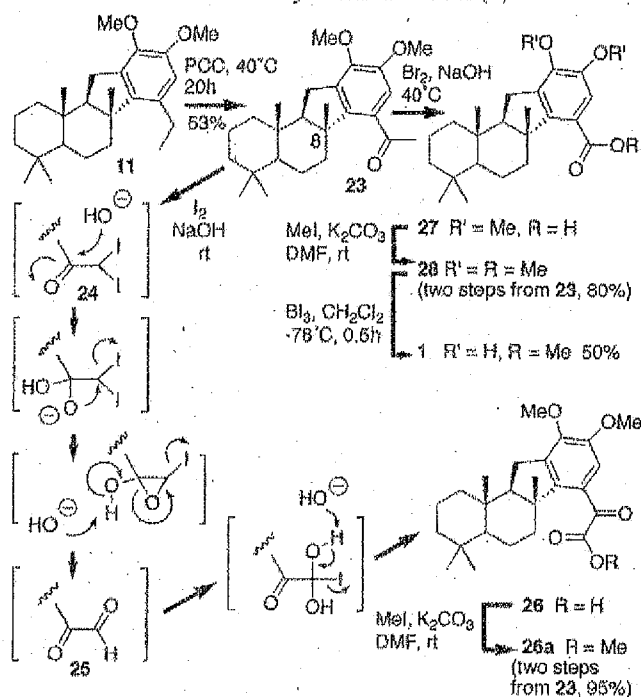
Sclareolide (**4**) was converted to the diol **14** in excellent yield (90%) using a one-pot three-step sequence modification of the literature procedure.<sup>16</sup> Swern oxidation cleanly oxidized the diol **14** to the aldehyde **15**.<sup>17</sup> Reaction of **15** with phenyllithium **7** gave the epimeric benzyl alcohols **16** in good yield. Hydrogenolysis of the mixture **16** cleanly removed the benzylic alcohols to give **17**. Cyclization of **17** using  $\text{SnCl}_4$  as a catalyst gave the desired tetracyclic intermediate **11** in high yield, without any trace of the elimination product **12**. Dimethyl ether **11** was converted to the catechol **21**, and the entire sequence was repeated with phenyllithium **7a** to give the methyl analogue **22** to provide polorol analogues for SAR.

Reaction of **11** with PCC selectively oxidized the C-22 methylene to give methyl ketone **23** in reasonable yield (Scheme 4). Treatment of **23** with  $\text{I}_2$  in aqueous  $\text{NaOH}$ , in

(16) Kuchkova, K. I.; Chumakov, Y. M.; Simonov, Y. A.; Bocelli, G.; Panasenkov, A. A.; Vlad, P. F. *Synthesis* **1997**, *9*, 1045–1048.

(17) Onura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651–1660.

Scheme 4. Synthesis of Pelorol (1)



an attempt to effect a haloform reaction to give benzoic acid 27, unexpectedly resulted in the near quantitative formation of the  $\alpha$ -ketoacid 26. This anomalous result might be caused by the steric bulk of the C-8 carbon ortho to the C-22 ketone preventing the formation of a triiodinated methyl. If a diiodinated methyl ketone 24 is attacked by hydroxide to give a tetrahedral intermediate, the diiodomethyl may not be a good enough leaving group to depart in the normal fashion to give a carboxylic acid. Instead, an intramolecular  $S_N2$  displacement of iodide can form an epoxide, which after fragmentation as shown in Scheme 4 can lead to the  $\alpha$ -keto aldehyde 25. Oxidation of 25 via iodination of the aldehyde hydrate can generate the final  $\alpha$ -ketoacid 26. Simply changing the halogen to  $Br_2$  led to a clean transformation of methyl ketone 23 to the desired benzoic acid 27.

The synthesis of pelorol (1) was completed by esterification of 27 with MeI followed by selective cleavage of the phenyl methyl ethers with  $Br_3$  at  $-78^\circ C$ . Synthetic pelorol (1) was identical by NMR and MS comparison with the natural product. The  $[\alpha]_D$  of the synthetic material was  $-64^\circ$  compared with values of  $-69^\circ$  reported by König and  $-71^\circ$  reported by Schmitz, confirming that the absolute configuration is 5S,8R,9R,10S as predicted by Schmitz.<sup>18</sup>

Pelorol (1), dimethylpelorol (28), the analogues 21 and 22, the corresponding methyl ethers 11 and 20, the trimethoxy pelorol analogue 10, and the uncyclized precursor 19 were tested for in vitro activation of SHIP and the ability to suppress degranulation and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) production in murine mast cells stimulated with IgE.<sup>18</sup> Synthetic pelorol (1), the ethyl analogue 21, and the methyl analogue 22 showed significant activity in all three assays, but the methyl ethers 10, 11, 19, 20, and 28 were inactive. The relative effectiveness of the active compounds in the SHIP activation assay was  $22 > 21 \approx 1$ , showing that replacement of the methyl ester at C-20 in pelorol with a methyl gives enhanced activity. Lack of activity in the dimethyl ethers 20, 11, and 28 demonstrates that at least one phenol is required for activity.

Compound 22 was chosen for further evaluation because it showed biological activity greater than that of pelorol and its synthesis was shorter. Side by side evaluation of 22's ability to suppress degranulation and TNF $\alpha$  release in SHIP $-/-$  and SHIP $+/+$  mast cells showed that it was only active in the SHIP $+/+$  cells, indicating that it selectively targets SHIP. Compound 22 also showed positive effects comparable to the reference standard dexamethasone in a standard mouse ear edema assay for topical antiinflammatory activity and in a mouse model of septic shock.<sup>18</sup>

In summary, the sponge meroterpenoid pelorol (1) has been identified as an activator of the inositol-5-phosphatase SHIP. An efficient synthesis of pelorol (1) and several analogues has confirmed the structure and absolute configuration of the natural product and provided preliminary SAR for the SHIP-activating pharmacophore. The C-20 methyl analogue 22 has shown promising in vivo activity in two mouse models of inflammation, supporting the initial hypothesis that selective small-molecule SHIP activators should represent a new class of antiinflammatory agents.

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**Supporting Information Available:** Experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(18) Pure compounds were tested at 5  $\mu g/mL$ . In the SHIP assay, 22 showed  $> 6$ -fold activation, whereas 1 and 21 showed ca. 2-fold activation. See Supporting Information for experimental details of the in vivo assays. A complete description of the biological activity of pelorol and the synthetic analogues will be published elsewhere.

## Supporting Information

### Synthesis of Pelorol and Analogs: Activators of the Inositol 5-Phosphatase SHIP

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Raymond J. Andersen<sup>\*†</sup>

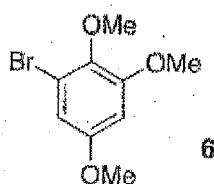
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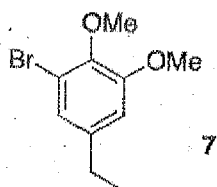
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## Experimental section

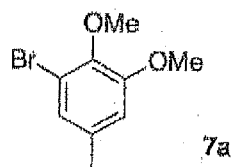
**General:** Starting materials and reagents were obtained from commercial sources and were used without further purification. Diethylether (Et<sub>2</sub>O) and tetrahydrofuran (THF) were purified by distillation from sodium/benzophenone immediately before use. Dichloromethane was distilled from CaH<sub>2</sub> under Argon. Moisture and oxygen sensitive reactions were carried out in flame-dried glassware under N<sub>2</sub>. Evaporations were conducted under reduced pressure at room temperature. Analytical thin layer chromatography was performed on silica gel 60 F-254 pre-coated aluminum plates (Merck). Column chromatography was carried out on 70-230 mesh silica gel and 10 g normal phase Sepaks with the indicated solvents. Proton and carbon nuclear magnetic resonance spectra were obtained using a Bruker Avance-400 spectrometer. High resolution electrospray ionization mass spectra (HRESIMS) were obtained on ESI micromass LCT spectrometer.



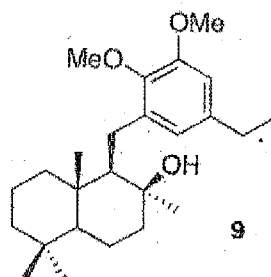
1-Bromo-2,3,5-trimethoxybenzene (6) was synthesized according to a literature procedure.<sup>1</sup>



**1-Bromo-2,3-dimethoxy-5-ethylbenzene (7).** 1-bromo-2,3-dimethoxy-5-ethenylbenzene was synthesized according to literature procedure.<sup>2</sup> Hydrogenation of 1-bromo-2,3-dimethoxy-5-ethenylbenzene (5.0 g, 20.4 mmol) was carried out overnight in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) under H<sub>2</sub> (3 bars) in the presence of 5% Rh/C (800 mg). The mixture was filtered and the catalyst was washed with acetone. The filtrate and acetone washings were combined, evaporated, and dried under high vacuum to afford 4.84 g of 7 (a colorless oil, 96%) which was used in the next step without purification. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.20 (t, J = 7.6 Hz, 3H), 2.55 (q, J = 7.6 Hz, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 6.66 (d, J = 1.8 Hz, 1H), 6.95 (d, J = 1.8 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 15.4, 28.5, 56.0, 60.5, 111.5, 117.3, 123.7, 141.4, 144.4, 153.4; HRESIMS calcd for C<sub>10</sub>H<sub>14</sub>O<sub>2</sub><sup>81</sup>Br 247.0157, found 247.0157.

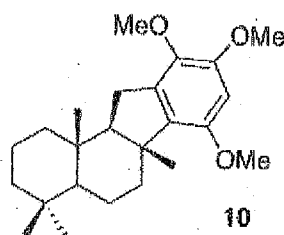


**1-Bromo-2,3-dimethoxy-5-methyl benzene (7a).**<sup>3</sup> To a stirred solution of 5-bromoveratraldehyde ( 2.0 g, 8.7 mmol) in 1,2-dichloroethane (45.0 mL) at ambient temperature were added zinc iodide ( 3.9 g, 12.2 mmol) and sodium cyanoborohydride ( 3.8 g, 61.2 mmol). The reaction mixture was heated under gentle reflux for 20 h and then filtered through celite. The filtrate was evaporated to dryness to afford 1.5 g of **7a** (light yellow oil, 80%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.26 (s, 3H), 3.80 (s, 3H), 3.82 (s, 3H), 6.63 (d, J = 1.4 Hz, 1H), 6.92 (d, J = 1.4 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 20.9, 56.0, 60.4, 112.6, 117.2, 124.9, 135.0, 144.2, 153.3; HRESIMS calcd for C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>Na<sup>81</sup>Br 254.9820, found 254.9830.

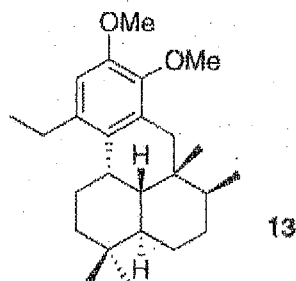


**Dimethylether (9).** A 1.7 M solution of t-BuLi in pentane (1.74 mL, 2.79 mmol) was added slowly to a stirred solution of **7** ( 627.4 mg, 2.54 mmol) in dry THF ( 20.0 mL) at -78°C. After stirring for 30 min, a solution of **5** (300mg, 1.26mmol) in dry THF (5.0 mL) was added. The mixture was further stirred at -78°C for 2 h after which H<sub>2</sub>O (10.0 mL) was added and the mixture was extracted with Et<sub>2</sub>O (120 mL, twice). The combined Et<sub>2</sub>O extracts were washed with saturated brine, dried (MgSO<sub>4</sub>), and concentrated to give a residue, which was chromatographed on a normal phase Sepak (8:2 Hexane/EtOAc) to give 306 mg of **9a** (white solid). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.87 (s, 3H), 0.88 (s, 3H), 1.03 (s, 3H), 1.21 (t, J = 7.6 Hz, 3H), 1.36 (s, 3H), 1.92 (brd, J = 12.5Hz, 1H), 2.59 (q, J = 7.7 Hz, 2H), 3.77 (s, 3H), 3.84 (s, 3H), 5.49 (s, 1H), 6.63 (d, J = 1.7Hz, 1H), 7.03 (d, J = 1.7 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 15.7, 16.3, 18.4, 18.8, 21.8, 29.0, 32.8, 33.5, 33.8, 39.3, 39.9, 41.9, 44.1, 55.6, 55.7, 59.1, 60.0, 69.5, 75.4, 76.7, 77.0, 77.3, 110.0, 118.9, 139.2, 139.8, 143.3, 151.9; HRESIMS calcd for C<sub>25</sub>H<sub>40</sub>O<sub>4</sub>Na 427.2824, found 427.2828. Hydrogenolysis of **9a** ( 202 mg, 0.5 mmol) was carried out overnight in EtOAc ( 8.0 mL) under H<sub>2</sub> (3 bars) in the presence of 10 % Pd/C (150 mg). The reaction mixture was filtered and concentrated to afford a brown oil, which was further purified by silica gel-column chromatography (9:1

Hexane/EtOAc) to give 165 mg of **9** (colorless oil, 85%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.85 (s, 3H), 0.87 (s, 3H), 0.90 (s, 3H), 1.06 (s, 3H), 1.21 (t,  $J = 7.6$  Hz, 3H), 1.73 (dt,  $J = 13.1, 2.7$  Hz, 1H), 1.79 (brd,  $J = 12.3$  Hz, 1H), 2.57 (q,  $J = 7.5$  Hz, 2H), 2.60 (dd,  $J = 15.7, 2.9$  Hz, 1H), 2.91 (dd,  $J = 15.7, 7.3$  Hz, 1H), 3.78 (s, 3H), 3.82 (s, 3H), 6.54 (d,  $J = 1.8$  Hz, 1H), 6.63 (d,  $J = 1.8$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  15.2, 15.8, 18.5, 18.5, 21.9, 23.4, 28.9, 31.6, 33.4, 33.6, 39.0, 40.0, 42.0, 42.9, 55.6, 56.0, 58.9, 60.6, 73.3, 109.0, 120.5, 137.9, 139.7, 144.5, 152.4; HRESIMS calcd for  $\text{C}_{25}\text{H}_{40}\text{O}_3\text{Na}$  411.2875, found 411.2880.

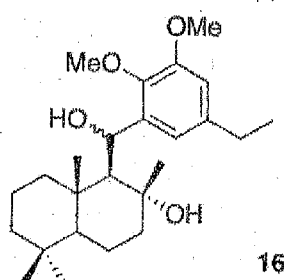


**Trimethylether (10).** The procedure for the preparation of **8** was the same as that used in the preparation of **9**. It afforded **8** (colorless oil, 20 mg, 60%), which was dissolved in 5 mL of  $\text{CH}_2\text{Cl}_2$  and  $\text{SnCl}_4$  (0.05 mL) was added slowly with stirring at  $-20^\circ\text{C}$  under argon for 2 min. The resulting mixture was further stirred for 20 min and then diluted with  $\text{CH}_2\text{Cl}_2$  (10 mL) and poured onto ice. The aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$  (two x 10 mL) and the combined extracts were washed with saturated  $\text{NaHCO}_3$ , saturated brine, and dried over  $\text{MgSO}_4$ . Evaporation of the  $\text{CH}_2\text{Cl}_2$  and purification of the residue by silica gel chromatography (40:1 Hexane/EtOAc) gave the trimethylether **10** (colorless oil, 14 mg, 70%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.84 (s, 3H), 1.01 (s, 3H), 1.06 (s, 3H), 1.23 (s, 3H), 1.71 (dd,  $J = 12.8, 6.1$  Hz, 1H), 2.40 (m, 1H), 2.50 (dd,  $J = 14.7, 12.9$  Hz, 1H), 2.69 (dd,  $J = 14.7, 6.2$  Hz, 1H), 3.74 (s, 3H), 3.76 (s, 3H), 3.82 (s, 3H), 6.28 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.2, 18.4, 19.5, 20.7, 21.1, 25.9, 33.1, 33.4, 37.0, 38.4, 40.2, 42.6, 46.8, 55.9, 56.3, 57.5, 60.6, 64.5, 96.3, 134.3, 136.8, 139.8, 150.9, 150.9; HRESIMS calcd for  $\text{C}_{24}\text{H}_{36}\text{O}_3\text{Na}$  395.2562, found 395.2564.

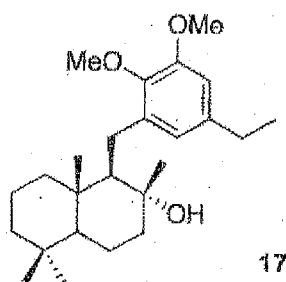


**Tetracycle (13).** Dimethylether **9** (100 mg, 0.26 mmol) was dissolved in 3.0 mL of polyphosphoric acid (PPA) and the mixture was heated under Argon at  $60^\circ\text{C}$  for 30 min. The resulting orange mixture was diluted with ice

water and extracted repeatedly with Et<sub>2</sub>O. The combined extracts were washed with brine and concentrated to give a brown oil, which was further purified by silica gel column chromatography (20:1 Hexane/EtOAc) to give 76.3 mg of **13** (80%). The structure of **13** was elucidated by analysis of COSY, HMBC, HMQC, and ROESY data. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.73 (s, 3H), 0.75 (s, 3H), 0.82 (s, 3H), 0.99 (d, J = 7.2 Hz, 3H), 1.21 (t, J = 7.5 Hz, 3H), 2.13 (d, J = 18.4 Hz, 1H), 2.61 (m, 2H), 3.08 (m, 1H), 3.20 (d, J = 18.4 Hz, 1H), 3.76 (s, 3H), 3.82 (s, 3H), 6.59 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.8, 15.9, 20.6, 21.6, 24.4, 26.3, 27.3, 28.8, 30.5, 32.1, 32.9, 33.7, 34.7, 37.6, 38.2, 38.9, 42.1, 55.5, 59.8, 111.4, 130.2, 132.0, 138.1, 144.4, 149.4; HRESIMS calcd for C<sub>25</sub>H<sub>38</sub>O<sub>2</sub>Na 393.2770, found 393.2768.

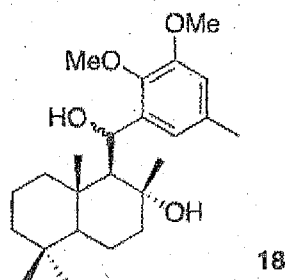


**Diol (16).** The procedure for the synthesis of **16** from **15** is the same as that used in the preparation of **9a** from **5** and it afforded **16** as a white solid (63%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.28 (td, J = 13.1, 3.4 Hz, 1H), 0.76 (s, 3H), 0.81 (s, 3H), 1.03 (s, 3H), 1.21 (t, J = 7.6 Hz, 3H), 1.54 (s, 3H), 1.85 (dt, J = 12.2, 3.2 Hz, 1H), 2.11 (d, J = 8.4 Hz, 1H), 2.60 (q, J = 7.6 Hz, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 5.25 (d, J = 8.4 Hz, 1H), 6.65 (d, J = 1.7 Hz, 1H), 6.90 (d, J = 1.7 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 15.7, 15.9, 18.4, 20.0, 21.7, 25.8, 28.9, 33.3, 33.6, 38.9, 40.0, 41.5, 44.7, 55.7, 56.0, 61.3, 62.7, 69.5, 74.7, 111.0, 119.6, 140.5, 140.6, 143.5, 152.3; HRESIMS calcd for C<sub>25</sub>H<sub>40</sub>O<sub>4</sub>Na 427.2824, found 427.2828.

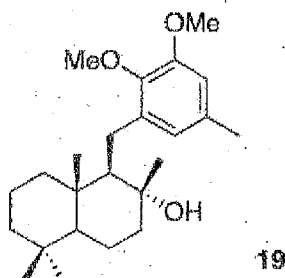


**Tertiary alcohol (17).** The procedure for the preparation of **17** from **16** is the same as that used in the preparation of **9** from **9a** and it afforded **17** as a colorless oil (87%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.78 (s, 3H), 0.83 (s, 3H), 0.89 (s, 3H), 1.08 (td, J = 13.3, 3.8 Hz, 1H), 1.19 (t, J = 7.6 Hz, 3H), 1.26 (s, 3H), 1.82 (dt, J = 12.5, 3.2 Hz,

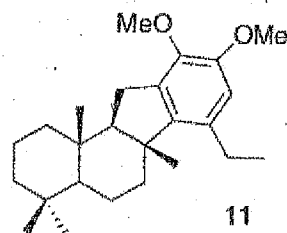
1H), 2.54 (q,  $J = 7.6\text{ Hz}$ , 2H), 2.81 (dd,  $J = 14.5, 5.5\text{ Hz}$ , 1H), 3.81 (s, 6H), 6.54 (d,  $J = 1.8\text{ Hz}$ , 1H), 6.64 (d,  $J = 1.8\text{ Hz}$ , 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  15.4, 15.7, 18.6, 20.3, 21.6, 24.3, 25.3, 28.8, 33.3, 33.5, 39.4, 40.3, 41.8, 43.7, 55.6, 56.1, 60.6, 62.8, 73.7, 109.6, 122.0, 137.6, 140.0, 144.3, 152.3; HRESIMS calcd for  $\text{C}_{25}\text{H}_{40}\text{O}_3\text{Na}$  411.2875, found 411.2871.



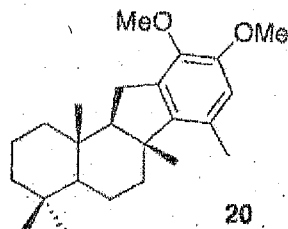
**Diol (18).** The procedure for the preparation of **18** from **15** was the same as that used in the preparation of **9a** from **5** and afforded **18** as a white solid (61%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.27 (td,  $J = 12.8, 3.1\text{ Hz}$ , 1H), 0.75 (s, 3H), 0.80 (s, 3H), 1.02 (s, 3H), 1.53 (s, 3H), 1.84 (dt,  $J = 12.2, 2.9\text{ Hz}$ , 1H), 2.10 (d,  $J = 8.5\text{ Hz}$ , 1H), 2.31 (s, 3H), 3.82 (s, 3H), 3.84 (s, 3H), 5.26 (d,  $J = 8.5\text{ Hz}$ , 1H), 6.61 (d,  $J = 1.5\text{ Hz}$ , 1H), 6.89 (d,  $J = 1.5\text{ Hz}$ , 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  15.8, 18.4, 20.0, 21.5, 21.6, 25.9, 33.2, 33.6, 38.8, 39.8, 41.5, 44.6, 55.6, 55.9, 61.4, 62.6, 69.0, 74.7, 112.1, 120.5, 134.1, 140.7, 143.3, 152.2; HRESIMS calcd for  $\text{C}_{24}\text{H}_{38}\text{O}_4\text{Na}$  413.2668, found 413.2664.



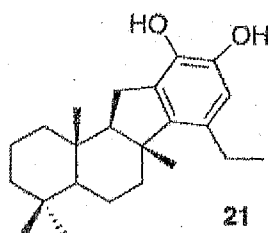
**Tertiary alcohol (19).** The procedure for the preparation of **19** from **18** was the same as that used in the preparation of **9** from **9a** and it afforded **19** as a colorless oil (89%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.78 (s, 3H), 0.83 (s, 3H), 0.88 (s, 3H), 1.27 (s, 3H), 1.76 (brd,  $J = 12.6\text{ Hz}$ , 1H), 1.82 (dt,  $J = 12.5, 3.2\text{ Hz}$ , 1H), 2.25 (s, 3H), 2.54 (dd,  $J = 14.5, 4.6\text{ Hz}$ , 1H), 2.78 (dd,  $J = 14.5, 5.6\text{ Hz}$ , 1H), 3.79 (s, 3H), 3.80 (s, 3H), 6.52 (d,  $J = 1.7\text{ Hz}$ , 1H), 6.62 (d,  $J = 1.7\text{ Hz}$ , 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  15.4, 18.6, 20.3, 21.4, 21.6, 24.3, 25.0, 33.3, 33.5, 39.4, 40.3, 41.8, 43.7, 55.6, 56.1, 60.6, 62.8, 73.7, 110.8, 123.1, 129.6, 133.6, 137.7, 152.3; HRESIMS calcd for  $\text{C}_{24}\text{H}_{38}\text{O}_3\text{Na}$  397.2719, found 397.2713.



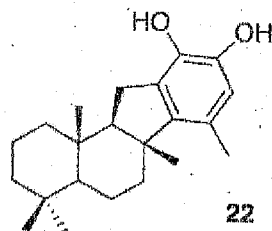
**Tetracycle (11).** To a stirred solution of **9** or **17** (38.8 mg, 0.1mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL),  $\text{SnCl}_4$  (0.1mL) was added slowly at  $-20^\circ\text{C}$  under argon for 2 min. The resulting mixture was stirred for a further 20 min and then diluted with  $\text{CH}_2\text{Cl}_2$  (20 mL) and poured onto ice. The aqueous phase was extracted with  $\text{CH}_2\text{Cl}_2$  (two x 20 mL) and the combined extracts were washed with saturated  $\text{NaHCO}_3$ , brine, and dried over  $\text{MgSO}_4$ . Evaporation of the  $\text{CH}_2\text{Cl}_2$  and purification of the residue by silica gel chromatography (20:1 Hexane/EtOAc) gave **11** as a colorless oil (29.5 mg, 76%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.85 (s, 6H), 1.02 (s, 3H), 1.08 (s, 3H), 1.22 (t,  $J = 7.6$  Hz, 3H), 2.36 (dt,  $J = 12.0, 3.4$  Hz, 1H), 2.49 (dd,  $J = 14.5, 13.0$  Hz, 1H), 2.56 (dd,  $J = 14.5, 7.5$  Hz, 1H), 2.69 (m, 2H), 3.80 (s, 3H), 3.81 (s, 3H), 6.49 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.0, 16.1, 18.3, 19.7, 21.1, 21.3, 24.7, 25.2, 33.1, 33.4, 37.1, 39.3, 40.2, 42.5, 47.9, 55.9, 57.1, 60.4, 64.3, 111.1, 133.7, 135.8, 143.6, 144.8, 150.3; HRESIMS calcd for  $\text{C}_{25}\text{H}_{38}\text{O}_2\text{Na}$  393.2770, found 393.2768.



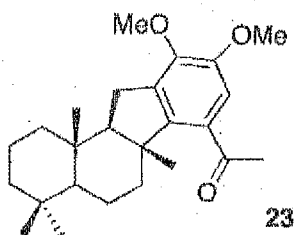
**Tetracycle (20).** The procedure for the preparation of **20** from **19** was the same as that used to prepare **11** from **17** and it afforded **20** as a colorless oil (85%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.85 (s, 6H), 1.02 (s, 3H), 1.06 (s, 3H), 2.24 (s, 3H), 2.32 (dt,  $J = 11.7, 3.1$  Hz, 1H), 2.49 (dd,  $J = 14.8, 13.0$  Hz, 1H), 2.69 (dd,  $J = 14.8, 6.1$  Hz, 1H), 3.47 (s, 6H), 6.41 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.1, 18.3, 18.6, 19.6, 20.5, 21.1, 25.3, 33.1, 33.4, 37.1, 38.8, 40.1, 42.5, 47.6, 55.9, 57.1, 60.4, 64.4, 113.0, 127.1, 135.8, 143.7, 145.2, 150.0; HRESIMS calcd for  $\text{C}_{24}\text{H}_{36}\text{O}_2\text{Na}$  379.2613, found 379.2614.



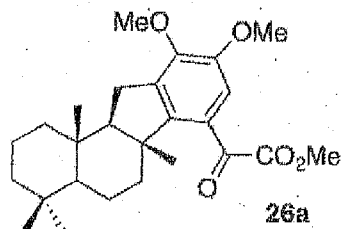
**Catechol (21).** To a stirred solution of **11** (38.6mg, 0.1mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) under Argon at 0 °C,  $\text{BBr}_3$  in  $\text{CH}_2\text{Cl}_2$  (2.0 mL 1M) was added and stirring was continued for 1.5 h at rt. The mixture was then poured into  $\text{H}_2\text{O}$  and extracted with  $\text{CH}_2\text{Cl}_2$  (three x 50 mL). The combined  $\text{CH}_2\text{Cl}_2$  extracts were dried over  $\text{MgSO}_4$ , filtered, and concentrated. The residue was purified by silica gel flash chromatography (hexane:EtOAc 7:3) to afford **21** as a white solid (32mg, 90%).  $^1\text{H}$ NMR ( $\text{CDCl}_3$ )  $\delta$  0.85 (s, 6H), 1.02 (s, 3H), 1.20 (s, 3H), 2.35 (dt,  $J$  = 12.2, 3.4 Hz, 1H), 2.43 (dd,  $J$  = 13.9, 13.6 Hz, 1H), 2.57 (m, 2H), 2.61 (dd,  $J$  = 14.5, 7.3 Hz, 1H), 6.47 (s, 1H);  $^{13}\text{C}$ NMR ( $\text{CDCl}_3$ )  $\delta$  15.9, 16.1, 18.3, 19.6, 21.0, 21.3, 24.1, 24.4, 33.0, 33.3, 37.0, 40.1, 42.4, 48.0, 57.0, 64.5, 113.5, 128.4, 130.8, 137.8, 141.3, 144.7; HRESIMS calcd for  $\text{C}_{23}\text{H}_{34}\text{O}_2\text{Na}$  365.2457, found 365.2456.



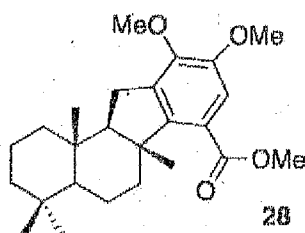
**Catechol (22).** The procedure for the preparation of **22** from **20** was the same as that used for the preparation of **21** from **11** and it gave **22** as a white solid (85%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.85 (s, 6H), 1.02 (s, 3H), 1.04 (s, 3H), 1.74 (dd,  $J$  = 12.6, 6.4 Hz, 1H), 2.17 (s, 3H), 2.31 (dt,  $J$  = 11.6, 3.0 Hz, 1H), 2.43 (dd,  $J$  = 14.0, 12.5 Hz, 1H), 2.57 (dd,  $J$  = 14.3, 6.4 Hz, 1H), 6.40 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.1, 18.2, 18.3, 19.5, 20.5, 21.1, 24.5, 33.0, 33.4, 37.0, 38.7, 40.0, 42.5, 47.8, 57.0, 64.6, 115.7, 124.1, 128.7, 138.0, 141.0, 145.2; HRESIMS calcd for  $\text{C}_{22}\text{H}_{32}\text{O}_2\text{Na}$  351.2300, found 351.2303.



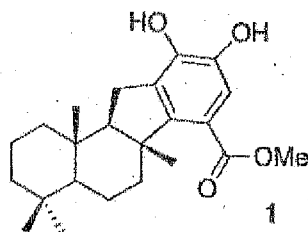
**Methylketone (23).** Pyridium chlorochromate (416 mg, 1.92 mmol) was added to **11** (74 mg, 0.2 mmol) dissolved in 20 mL of  $\text{CH}_2\text{Cl}_2$ . The mixture was stirred at gentle reflux for 20 h under Argon. After cooling to rt, the reaction was diluted with  $\text{Et}_2\text{O}$  (80 mL) and the resulting dark solution was filtered through a pad of silica gel. Concentration of the filtrates and purification of the residue via silica gel chromatography (9:1 Hexane/ $\text{EtOAc}$ ) afforded 40.7 mg (53 %) of **23** as a colorless oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.82 (s, 3H), 0.84 (s, 3H), 1.03 (s, 3H), 1.22 (s, 3H), 2.31 (dt,  $J = 12.5, 3.7\text{Hz}$ , 1H), 2.49 (s, 3H), 2.54 (dd,  $J = 14.9, 12.5\text{Hz}$ , 1H), 2.69 (dd,  $J = 14.9, 6.7\text{Hz}$ , 1H), 3.84 (s, 3H), 3.86 (s, 3H), 6.79 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.2, 18.3, 19.4, 20.5, 21.1, 25.2, 30.2, 33.1, 33.4, 36.4, 37.1, 40.2, 42.5, 48.0, 56.2, 57.2, 60.4, 64.9, 110.1, 130.5, 137.4, 147.6, 147.9, 149.6, 201.8; HRESIMS calcd for  $\text{C}_{25}\text{H}_{36}\text{O}_3\text{Na}$  407.2562, found 407.2563.



**Ketoester (26a).** Methylketone **23** (15 mg, 0.04 mmol) was dissolved in 20 mL of stirring NaOH (10%) solution (containing 5 mL THF). Iodine (50 mg) was added and the mixture was stirred for an additional 20 min before being acidified by addition of 30 mL of 10%  $\text{H}_2\text{SO}_4$ . The solution was extracted with 150 mL of  $\text{Et}_2\text{O}$ , the  $\text{Et}_2\text{O}$  was washed with brine and then concentrated to dryness to afford a residue, which was dissolved in 10 mL of DMF. Potassium carbonate (0.5g) and MeI (1.0 mL) were added to the DMF solution and stirring was continued overnight. The reaction was quenched by adding 10 mL of brine and the resulting mixture was extracted with  $\text{Et}_2\text{O}$ . The  $\text{Et}_2\text{O}$  was washed with brine, dried with  $\text{MgSO}_4$ , and evaporated to dryness. The residue was purified by silica gel chromatography (9:1 Hexane/ $\text{EtOAc}$ ) to give 15 mg of **26a** (95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.84 (s, 3H), 0.87 (s, 3H), 1.19 (s, 3H), 1.41 (s, 3H), 1.79 (dd,  $J = 11.9, 7.8\text{Hz}$ , 1H), 2.41 (brd,  $J = 11.9\text{Hz}$ , 1H), 2.74 (dd,  $J = 15.7, 12.5\text{Hz}$ , 1H), 2.93 (dd,  $J = 15.7, 8.1\text{Hz}$ , 1H), 3.81 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 6.93 (s, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  18.4, 18.5, 21.9, 24.2, 24.5, 30.1, 32.9, 33.5, 36.6, 36.7, 38.4, 42.4, 47.9, 48.1, 52.8, 53.4, 56.2, 60.4, 63.2, 114.1, 118.2, 125.3, 136.4, 149.4, 150.6, 164.6, 187.4; HRESIMS calcd for  $\text{C}_{26}\text{H}_{36}\text{O}_5\text{Na}$  451.2460, found 451.2457.



**Methylester (28).** A solution of 0.21 g of sodium hydroxide in 2.0 mL of water was added to 0.15 mL of bromine at 0 °C over 10 minutes. To this solution was added 38 mg (0.1 mmol) of **23** in 1 mL of dioxane. After stirring at 40 °C for 30 min, the yellow solution was quenched by addition of sodium bisulfite. Following acidification with 10% H<sub>2</sub>SO<sub>4</sub>, the mixture was extracted with Et<sub>2</sub>O. The Et<sub>2</sub>O was washed with brine and dried to furnish a residue, which was dissolved in 10 mL of DMF. Potassium carbonate (1.0 g) and MeI (2.0 mL) were added to the DMF solution and stirring was continued overnight. The reaction was quenched by adding 20 mL of brine and the resulting mixture was extracted with Et<sub>2</sub>O. The Et<sub>2</sub>O was washed with brine, dried with MgSO<sub>4</sub>, and evaporated to dryness. The residue was purified by silica gel column chromatography (9:1 Hexane/EtOAc) to give 32 mg of **28** (80%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.83 (s, 3H), 0.84 (s, 3H), 1.04 (s, 3H), 1.22 (s, 3H), 2.44 (dt, J = 12.3, 3.4 Hz, 1H), 2.54 (dd, J = 14.3, 12.9 Hz, 1H), 2.69 (dd, J = 14.3, 6.3 Hz, 1H), 3.82 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 7.00 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 16.3, 18.3, 19.5, 19.9, 21.1, 25.2, 33.1, 33.3, 36.5, 37.1, 40.2, 42.5, 48.1, 51.7, 56.1, 57.1, 60.4, 64.8, 111.6, 121.2, 137.0, 148.3, 148.7, 149.8, 168.3; HRESIMS calcd for C<sub>25</sub>H<sub>36</sub>O<sub>4</sub>Na 423.2511, found 423.2513.



**(-) Pelorol (1).** To a solution of methyl ester **28** (5 mg, 0.013 mmol) in 2 mL of CH<sub>2</sub>Cl<sub>2</sub> at -78 °C under Argon was slowly added BI<sub>3</sub> (60 mg) in 2 mL of CH<sub>2</sub>Cl<sub>2</sub> and mixture stirred for an additional 0.5 h. The mixture was then poured into H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (two x 50 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over MgSO<sub>4</sub>, filtered, and concentrated. The residue was purified by silica gel flash chromatography (4:1 Hexane/EtOAc) to furnish **1** (2.3 mg, 50%). [α]<sub>D</sub><sup>22.8</sup> = -63.5 (0.06, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.83 (s, 3H), 0.84 (s, 3H), 0.97 (td, J = 12.8, 3.7 Hz, 1H), 1.04 (s, 3H), 1.21 (s, 3H), 1.66 (dd, J = 12.5, 6.4 Hz, 1H), 2.48 (dd, J = 14.6, 12.6 Hz, 1H), 2.51 (m, 1H), 2.60 (dd, J = 14.3, 6.4 Hz, 1H), 3.81 (s, 3H), 7.07 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)

δ 16.3, 18.4, 19.5, 19.8, 21.1, 24.3, 33.0, 33.3, 36.5, 37.1, 40.2, 42.5, 48.5, 51.7, 57.1, 65.2, 114.8, 118.2, 130.0, 140.5, 143.4, 149.5, 168.0; HRESIMS calcd for  $C_{23}H_{32}O_4Na$  395.2198, found 395.2199.

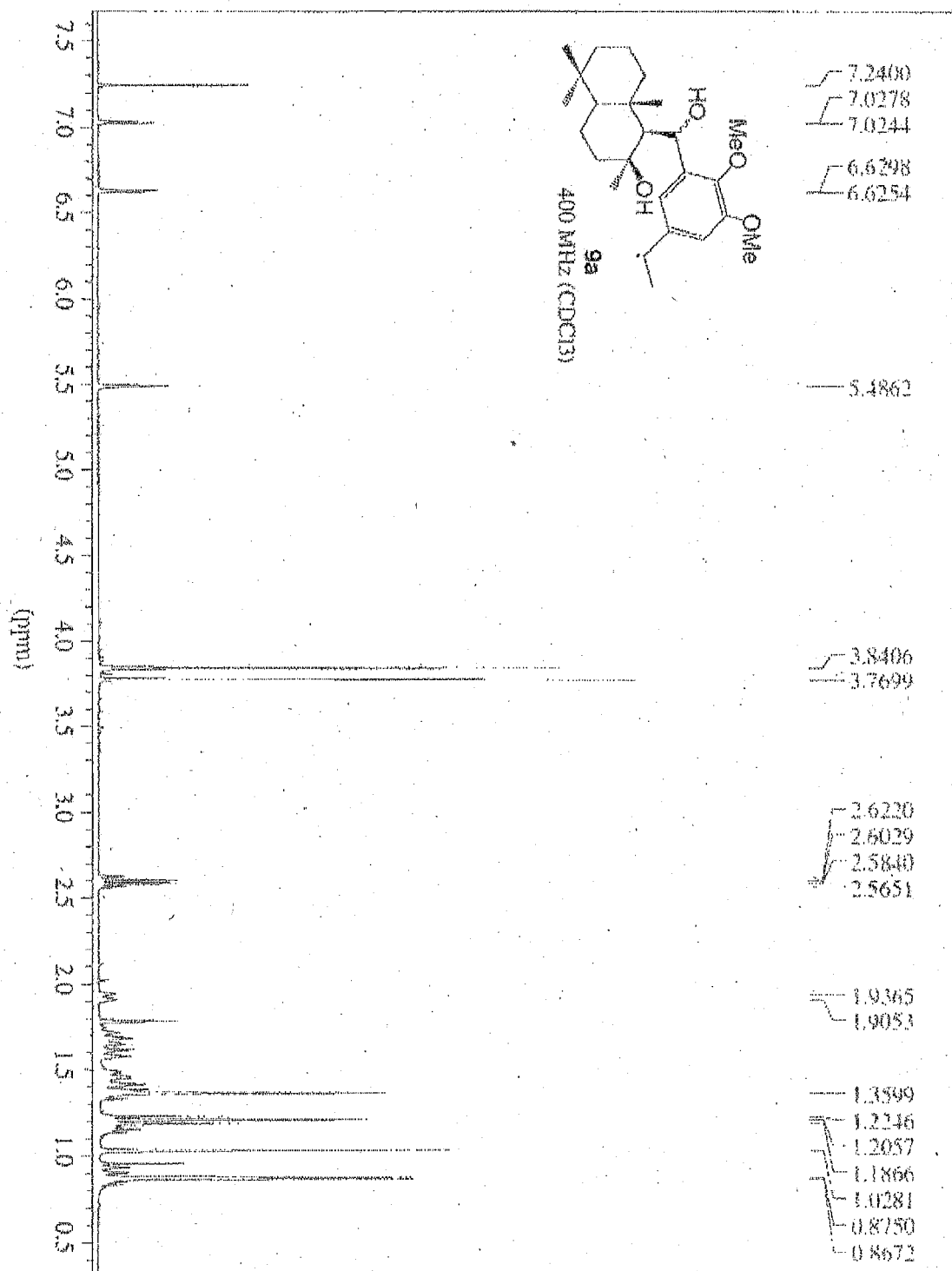
**Protocol for Evaluation of Compound 22 in a Mouse Septicemia Model.** Administration of the bacterial cell wall product, LPS (lipopolysaccharide), into mice results in rapid activation of macrophages to produce pro-inflammatory mediators such as  $TNF\alpha$  that can be detected circulating in the blood. This is a good model of human sepsis due to bacterial infection, and can be used to assess the ability of test compounds to inhibit macrophage-dependent inflammatory processes. Mice are administered the test compound (either intra-peritoneally or orally) 30 min prior to an intra-peritoneal injection of 20 mg/kg of LPS. Blood is drawn 2 hr later for determination of plasma  $TNF\alpha$  by ELISA.

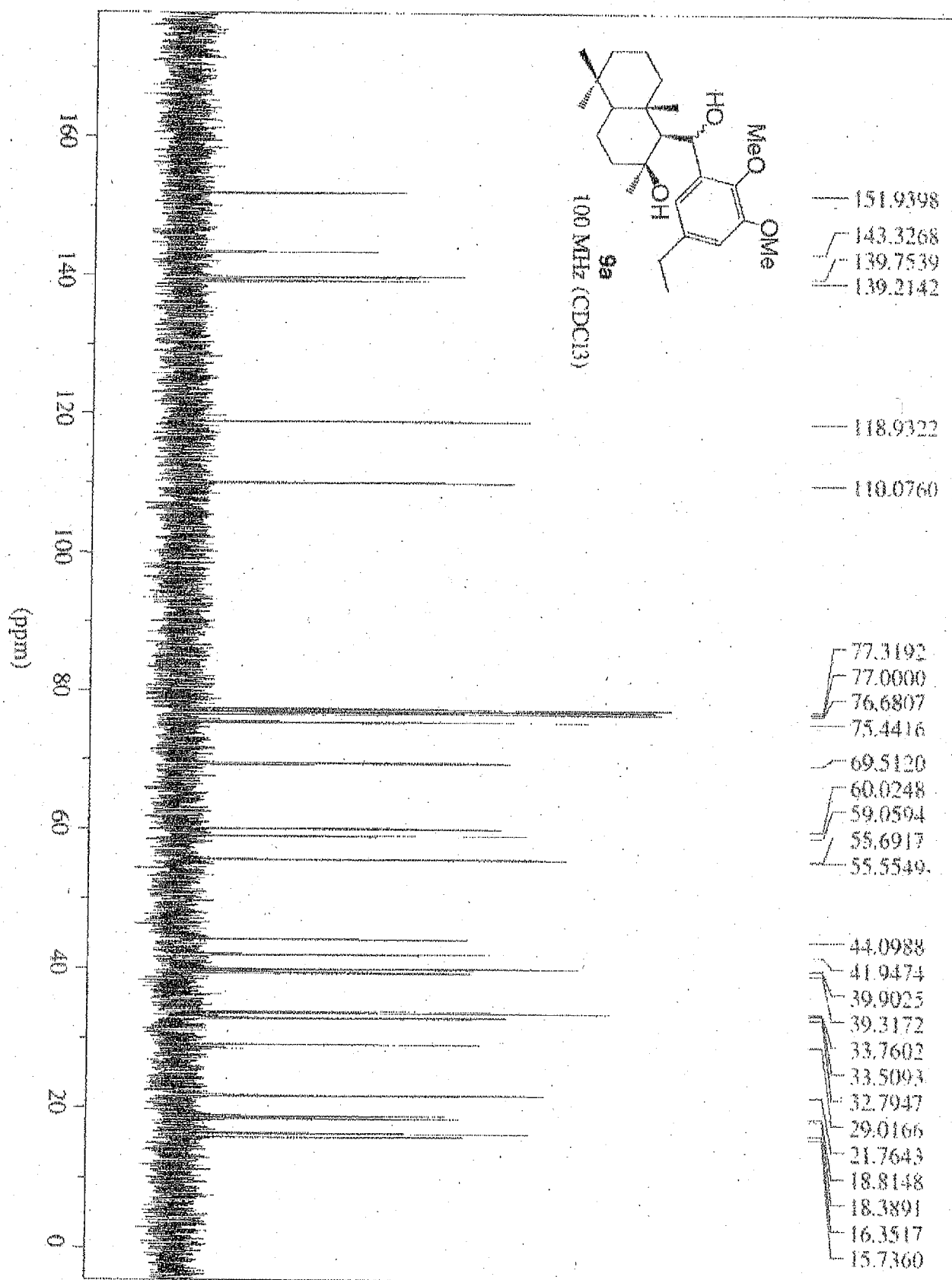
**Protocol for Evaluation of Compound 22 in a Mouse Acute Anaphylaxis Model.** The cutaneous anaphylaxis (or ear edema) model is a standard model of IgE (immunoglobulin E) and mast cell-dependent allergic inflammation. Mice are sensitized to the hapten DNFB (dinitrofluorobenzene) by cutaneous application of DNFB to the shaved abdomen of mice for two consecutive days. One week after the first application, tritiated thymidine (3H-Tdr) is injected intra-peritoneally. 24 hr later, test substances are painted on the left ear while the right ear receives vehicle control. 30 min after drug application, DNFB is applied to both ears to induce mast cell degranulation. This results in edema and neutrophil infiltration that can be quantitated by taking a 6 mm diameter punch from the ear for dissolution and scintillation counting. The ability of test substances to inhibit mast cell degranulation is then determined by calculating the ratio of 3H-Tdr in the test (right) ear vs the control (left) ear. One group of mice has DNFB applied only to the left ear leaving the right ear non-inflamed, in order to control for basal 3H-Tdr incorporation into ear parenchymal cells.

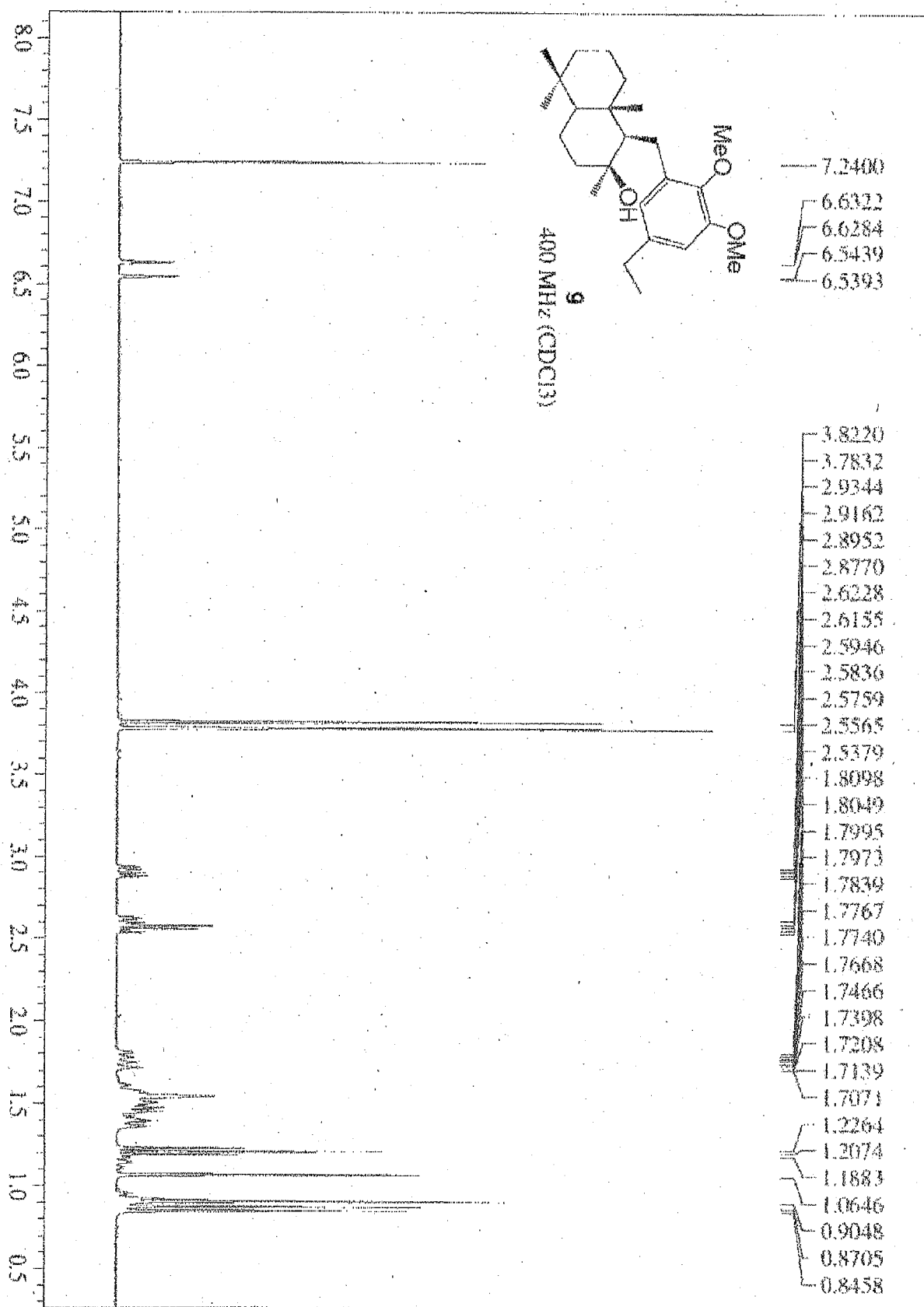
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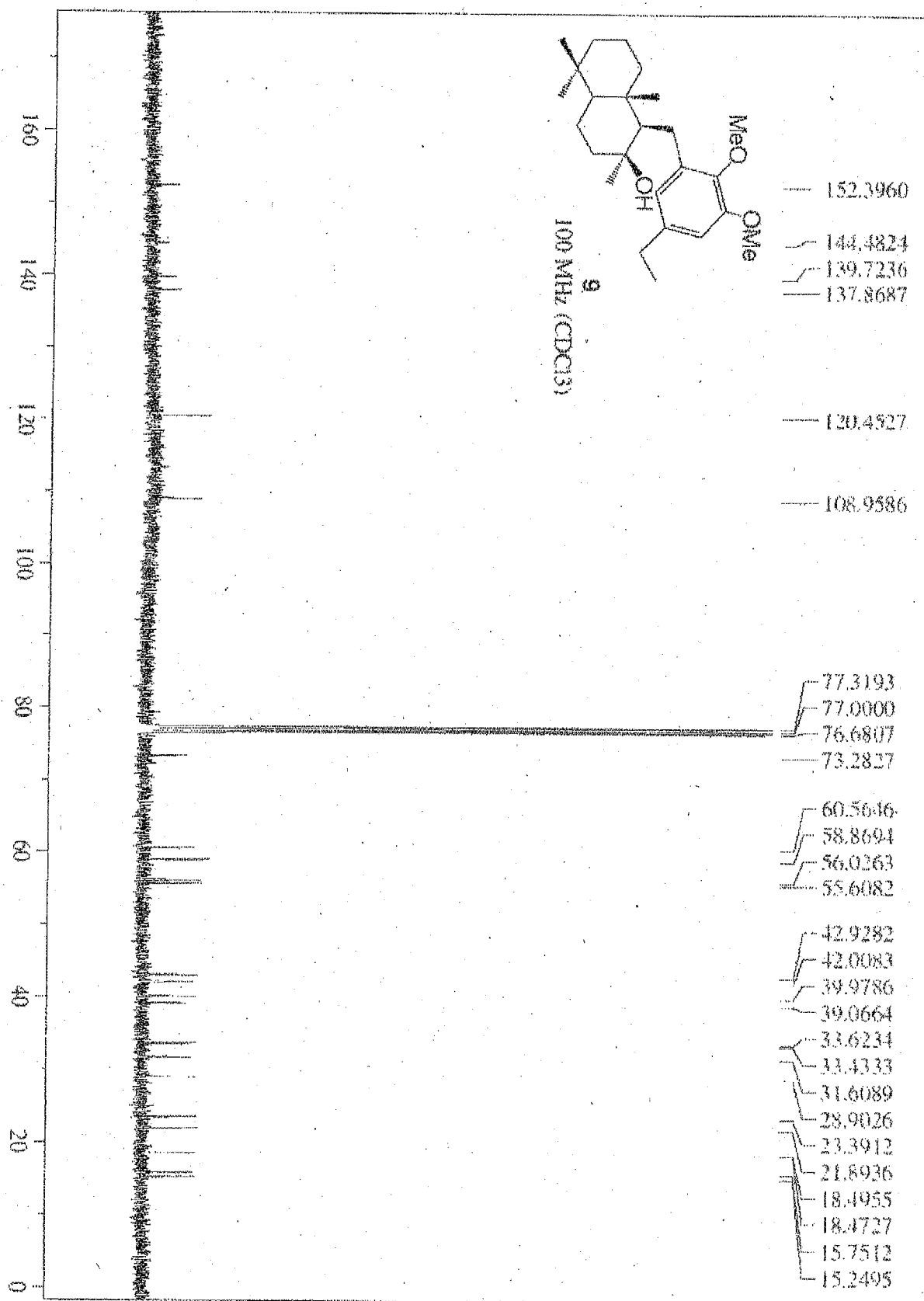
- (1) Sánchez, I.H.; Larraza, M.I.; Basurto, F.; Yañez, R.; Avila, S.; Tovar, R.; Nathan, P.J. *Tetrahedron*, **1985**, *41*, 2355-9.
- (2) Findlay, J.A.; Daljeet, A.; Murray, P.J.; Rej, R.N. *Can. J. Chem.* **1987**, *65*, 427-431.
- (3) Lau, C.K.; Dufresne, C.; Belanger, P.C.; Pietre, S.; Scheigetz, J. *J. Org. Chem.* **1986**, *51*, 3038-3043.

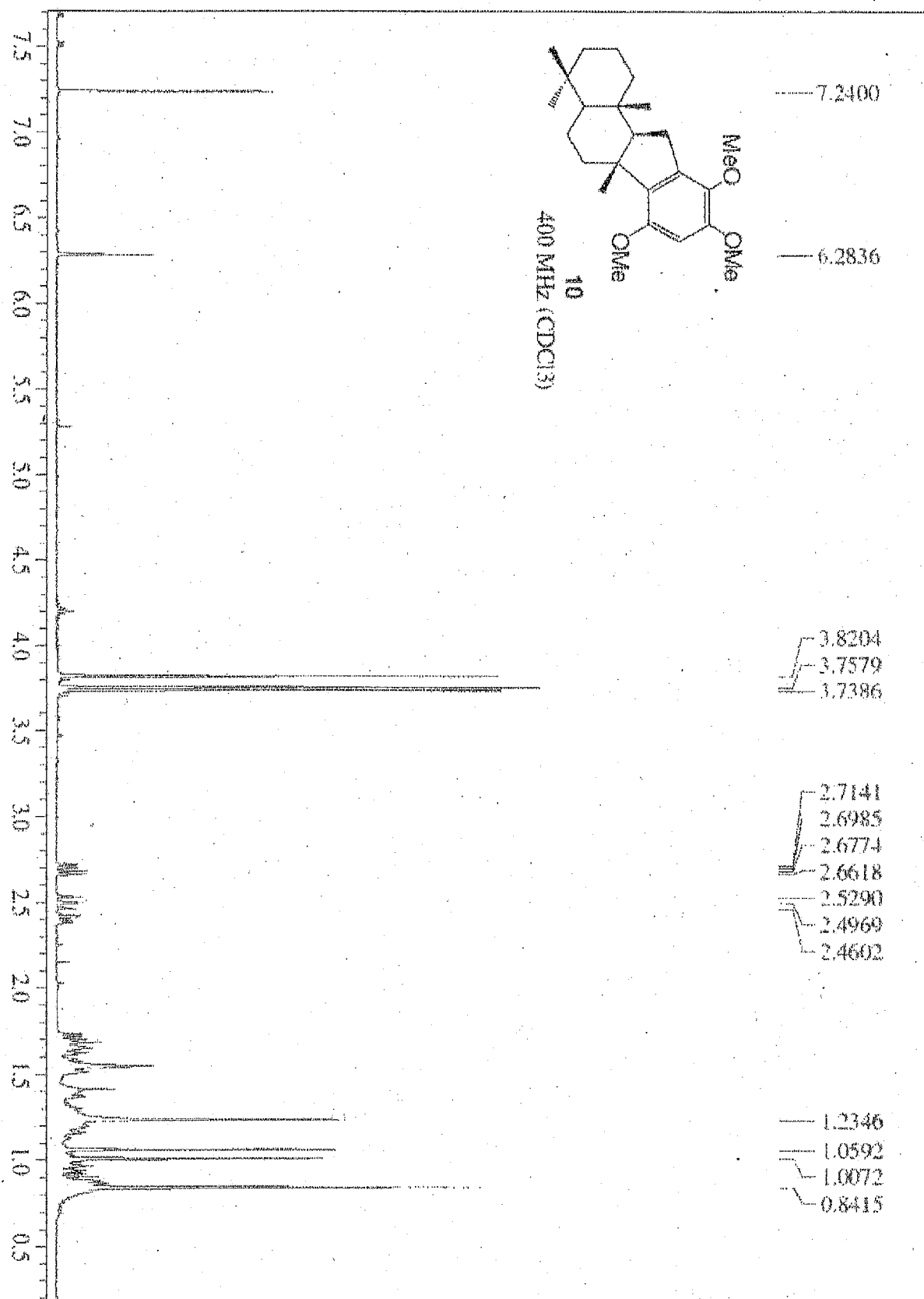
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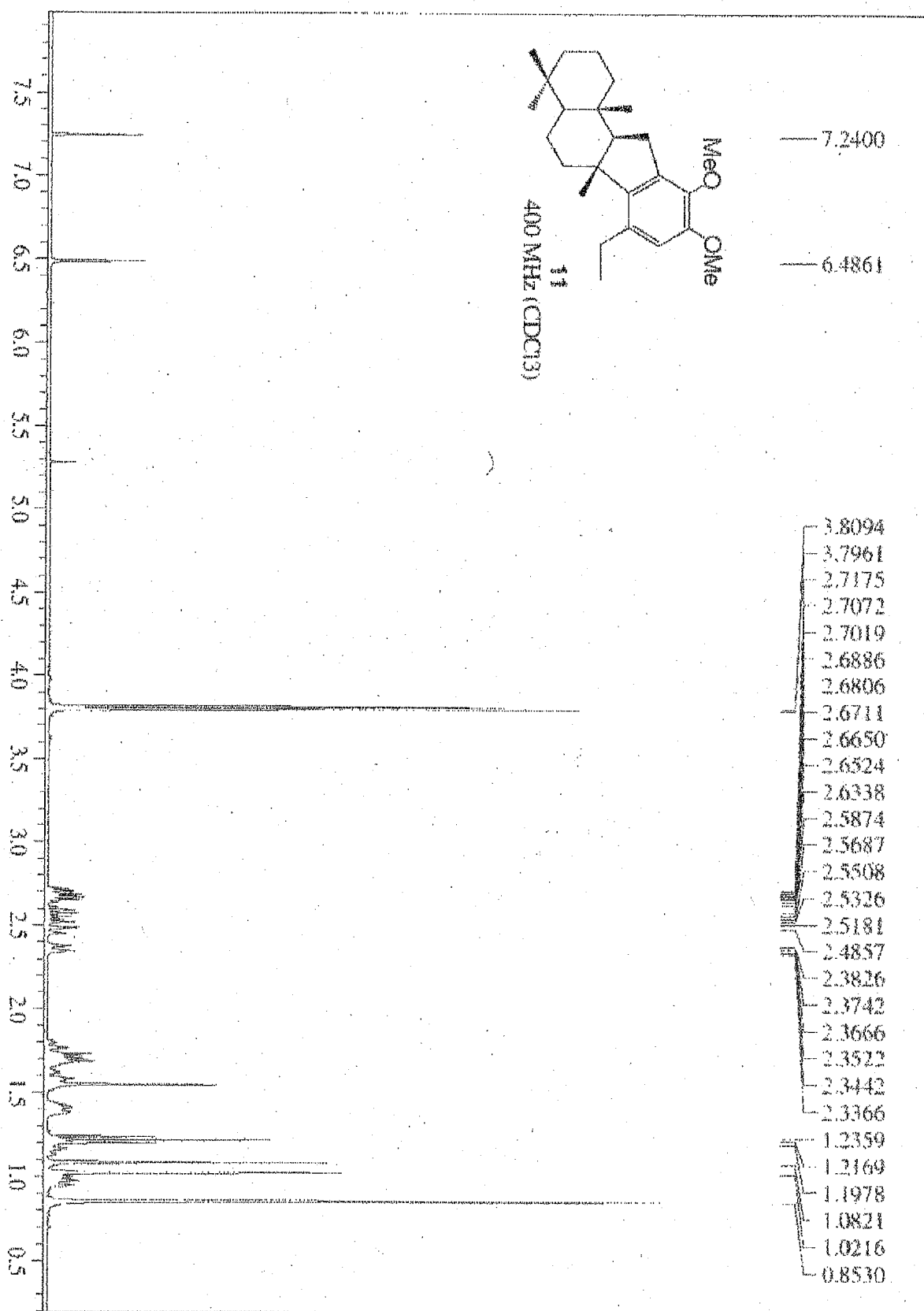


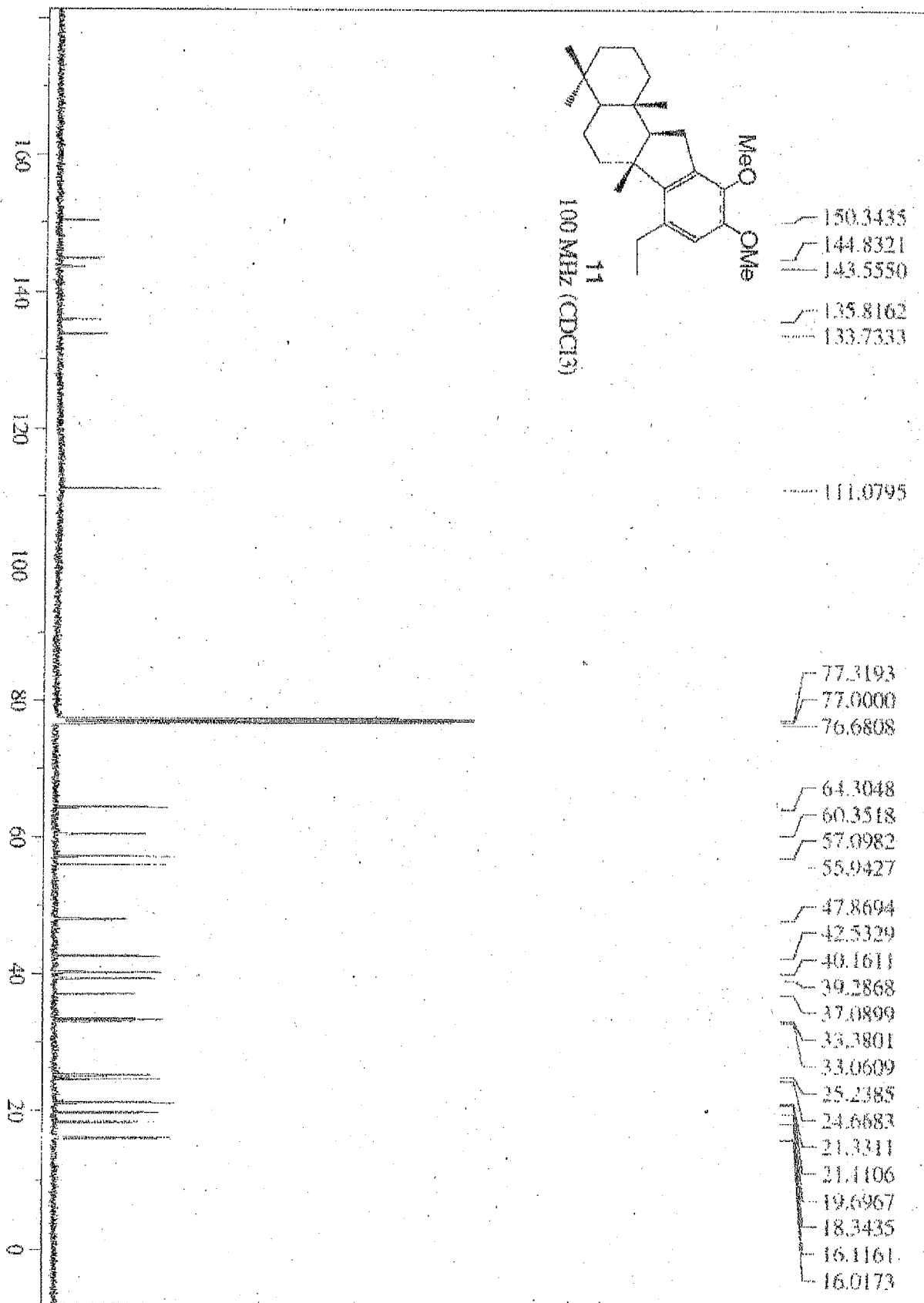


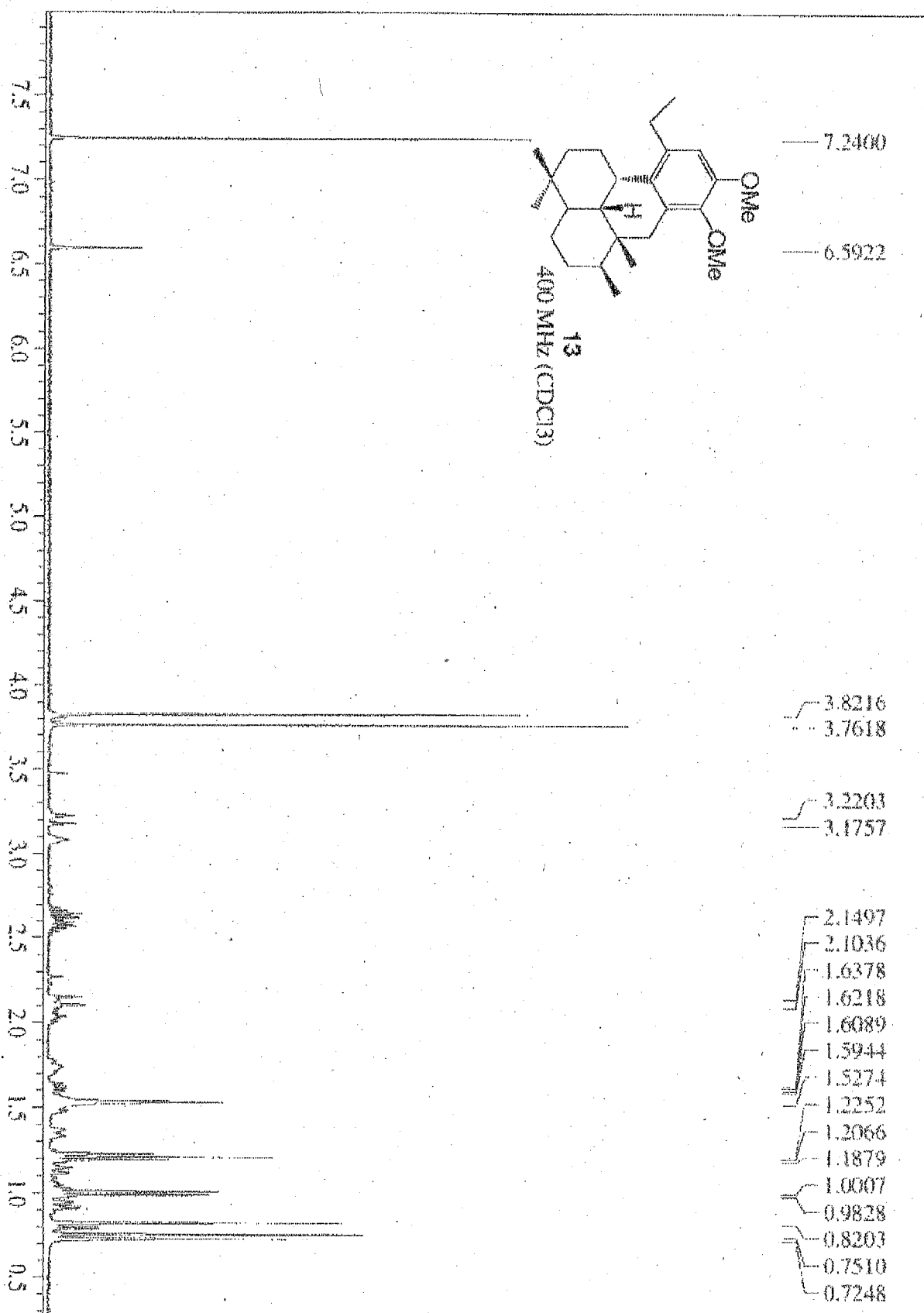


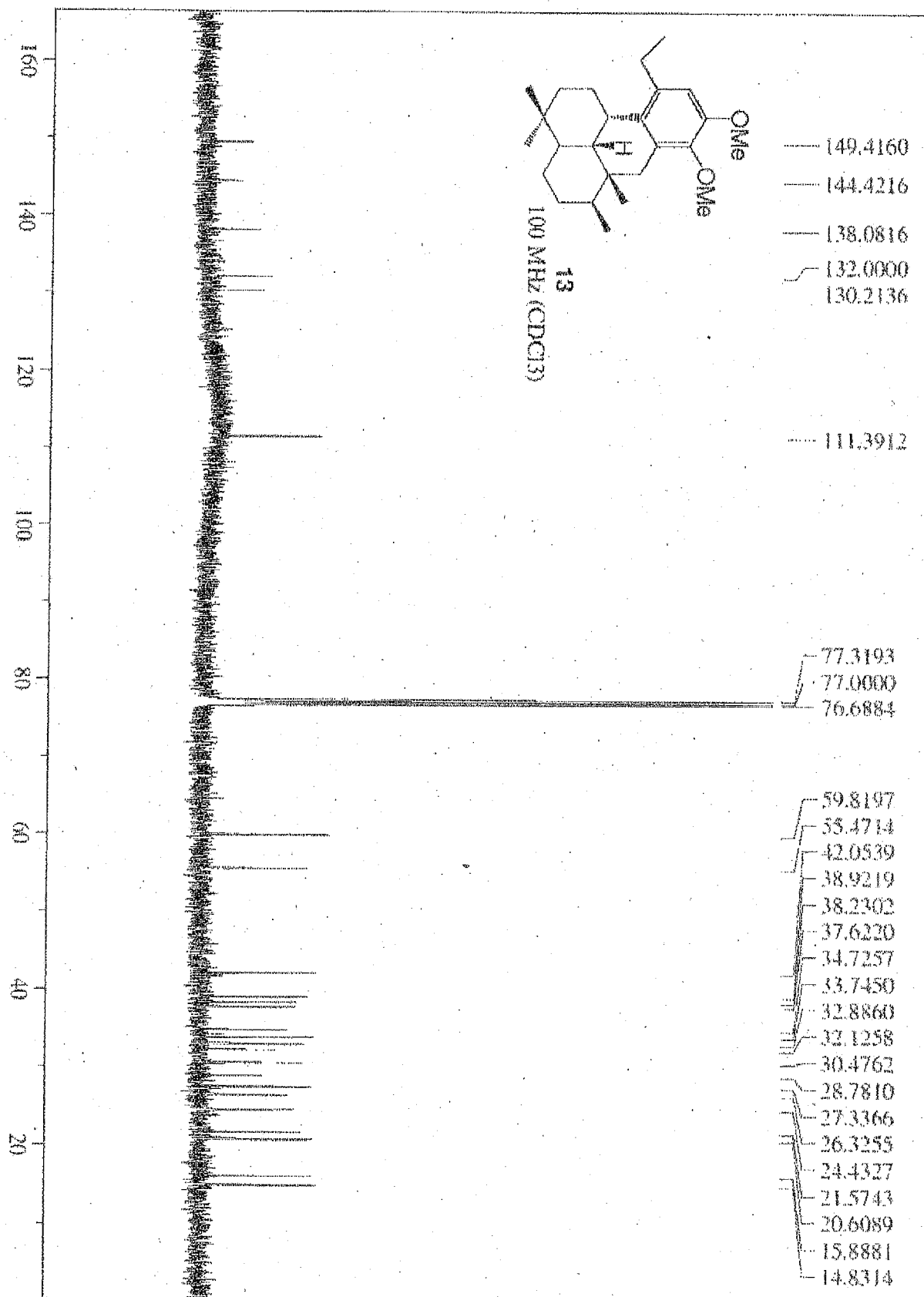




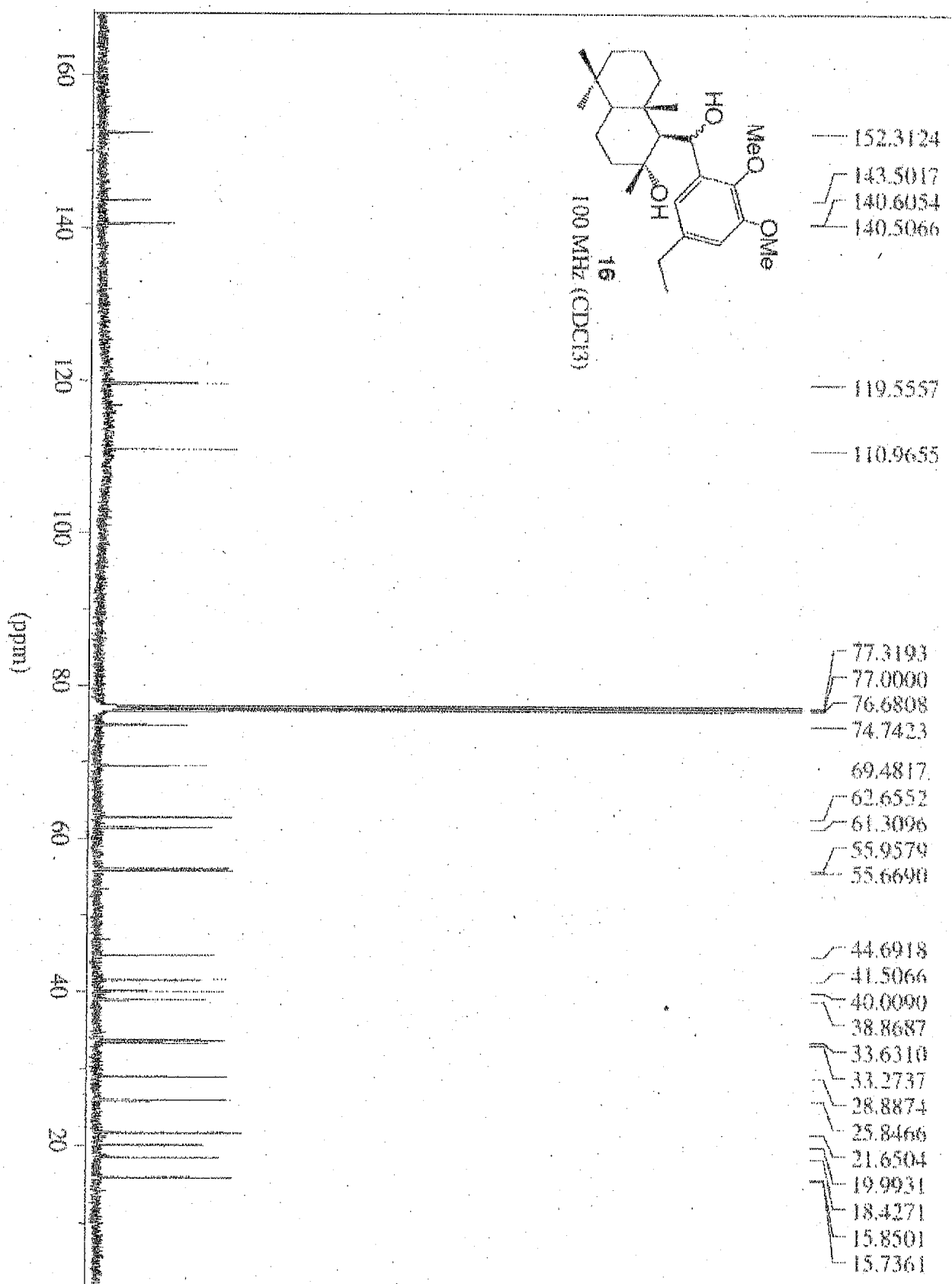


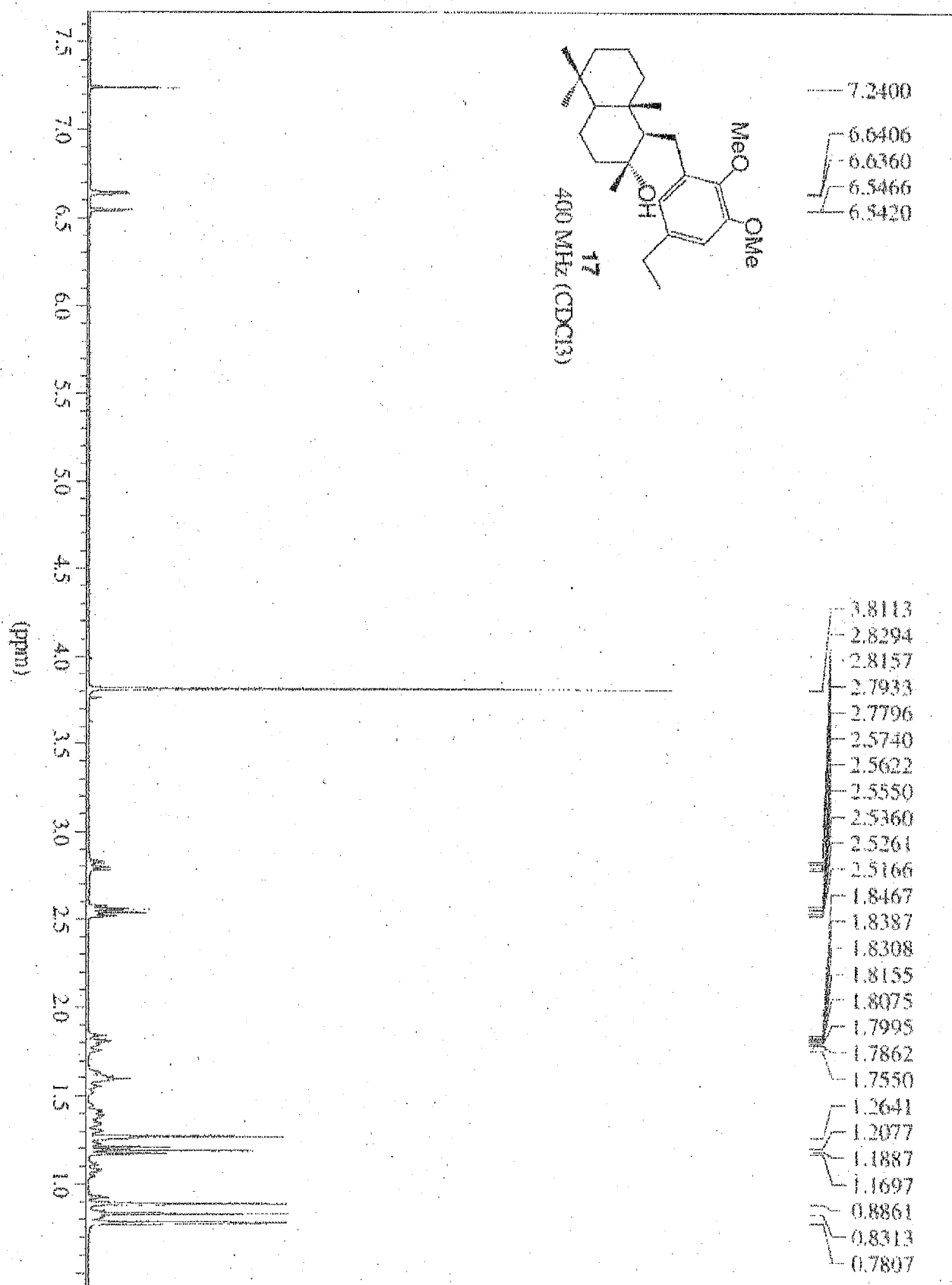


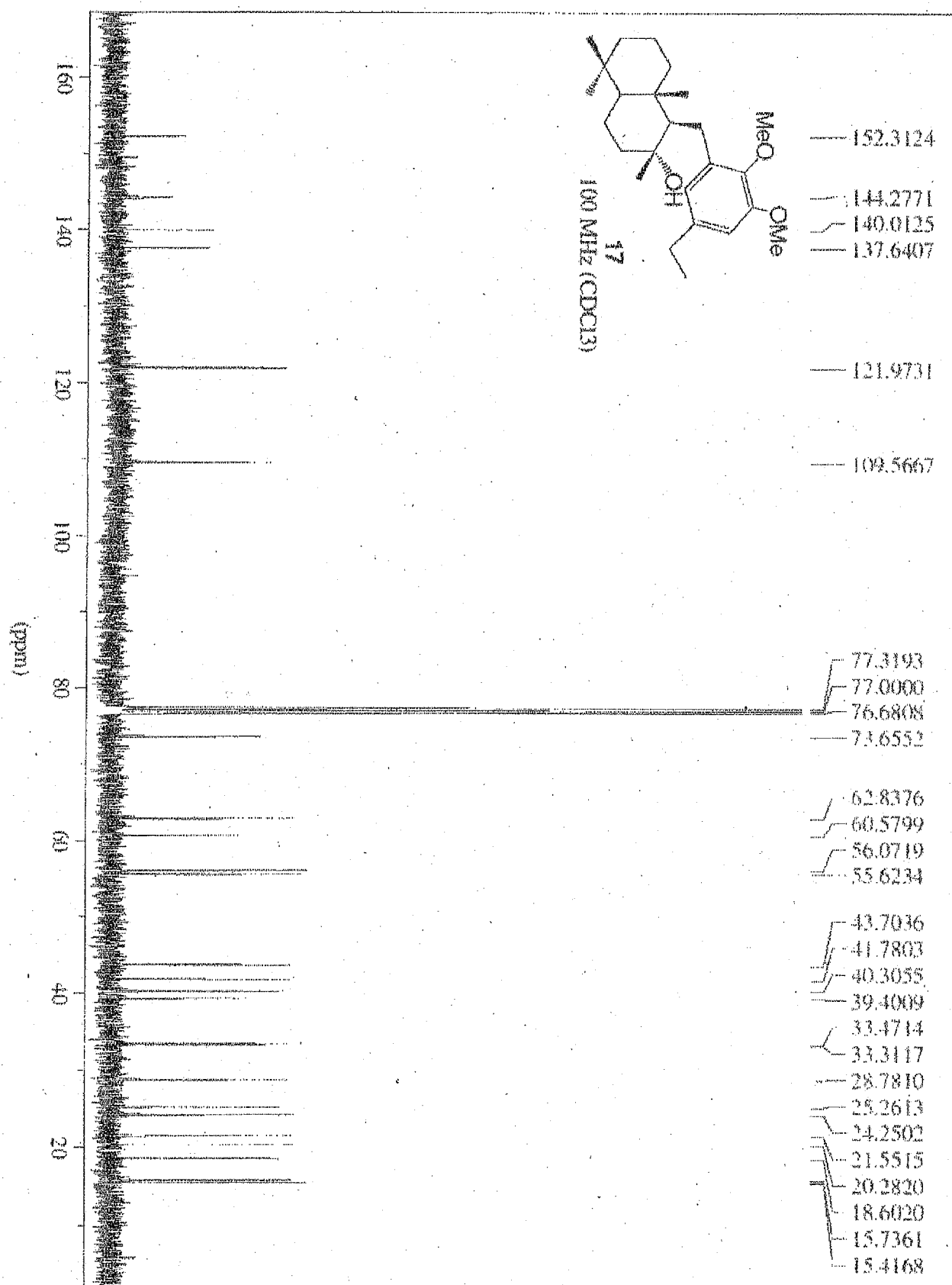


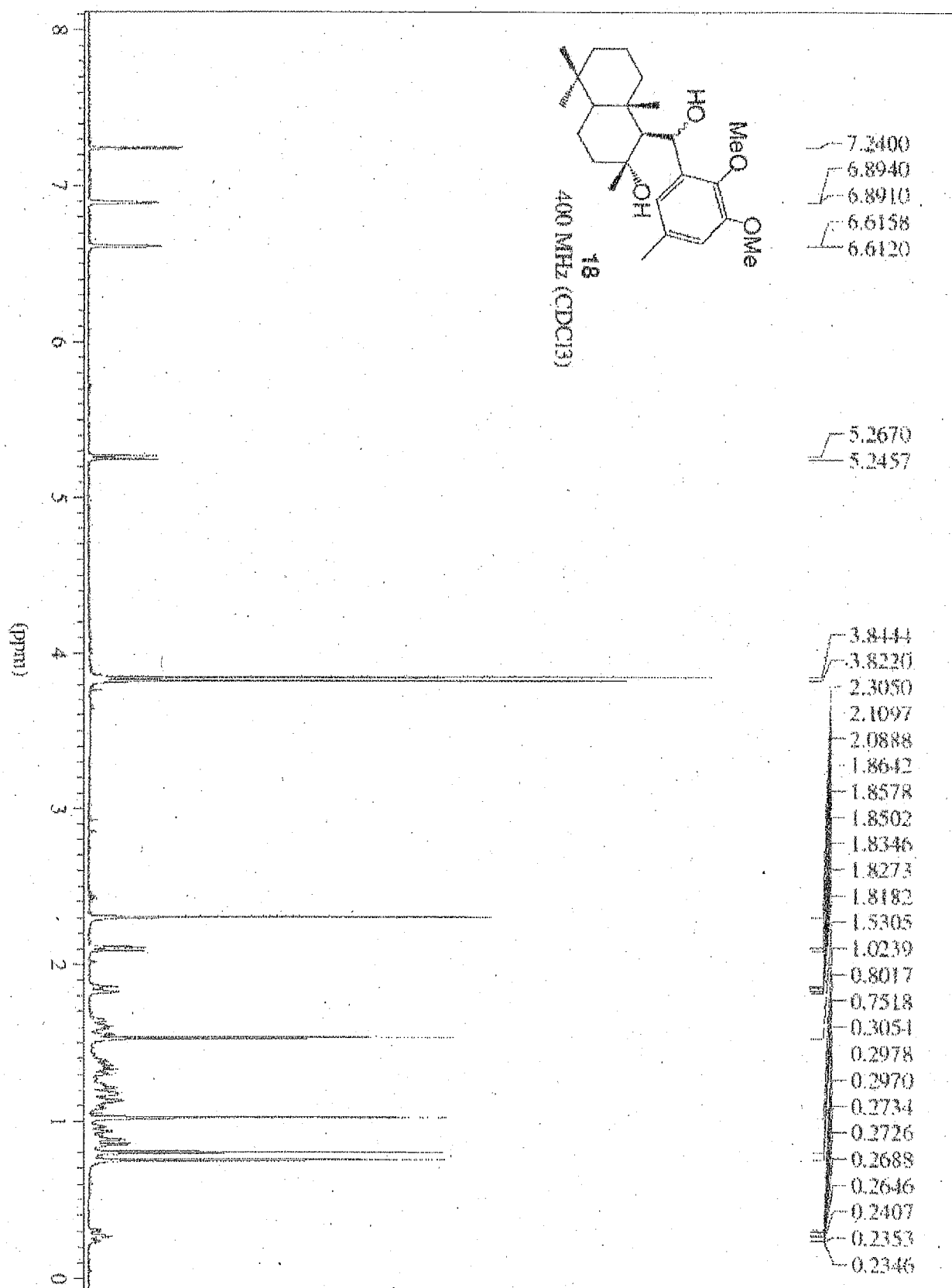


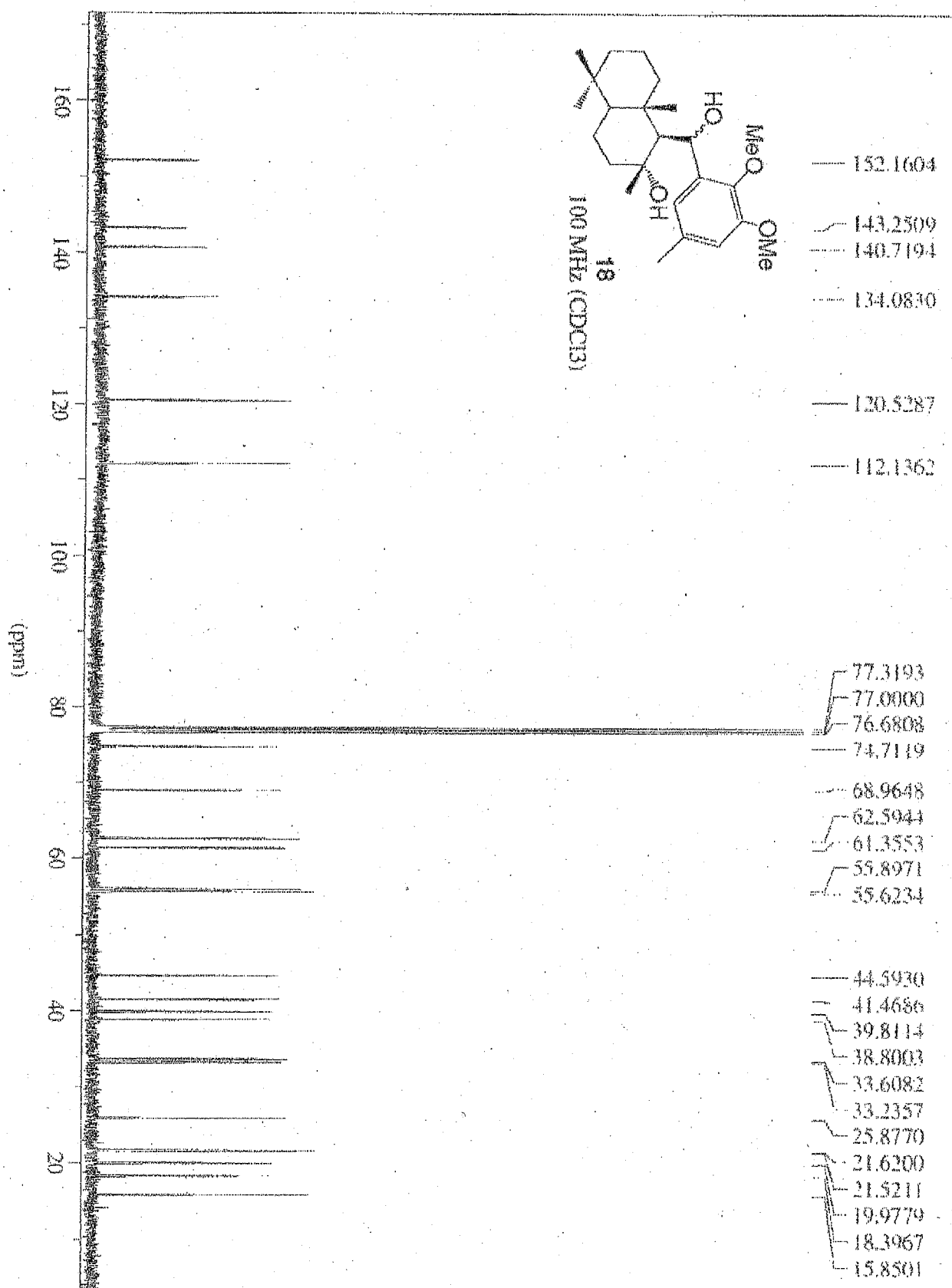


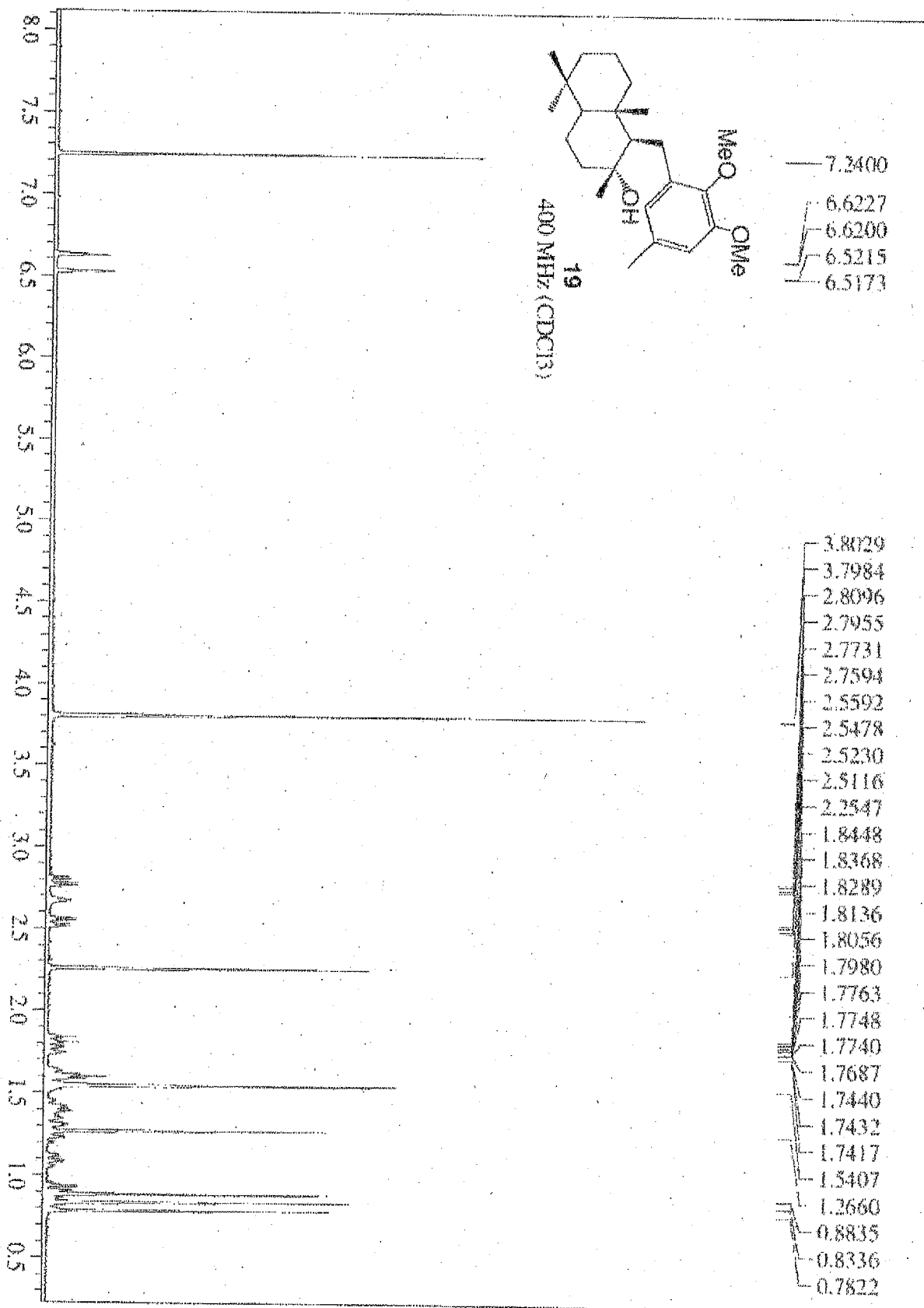


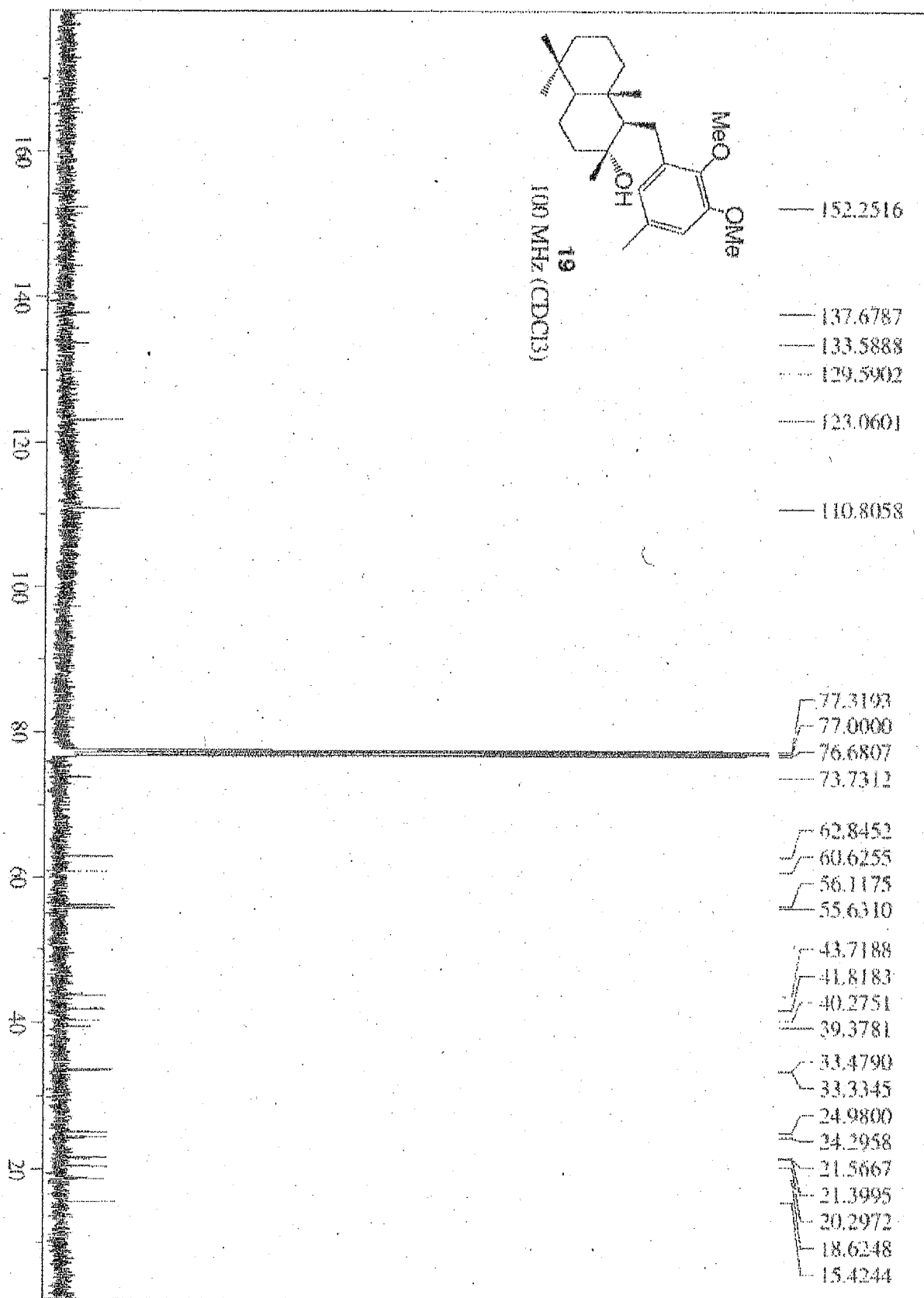


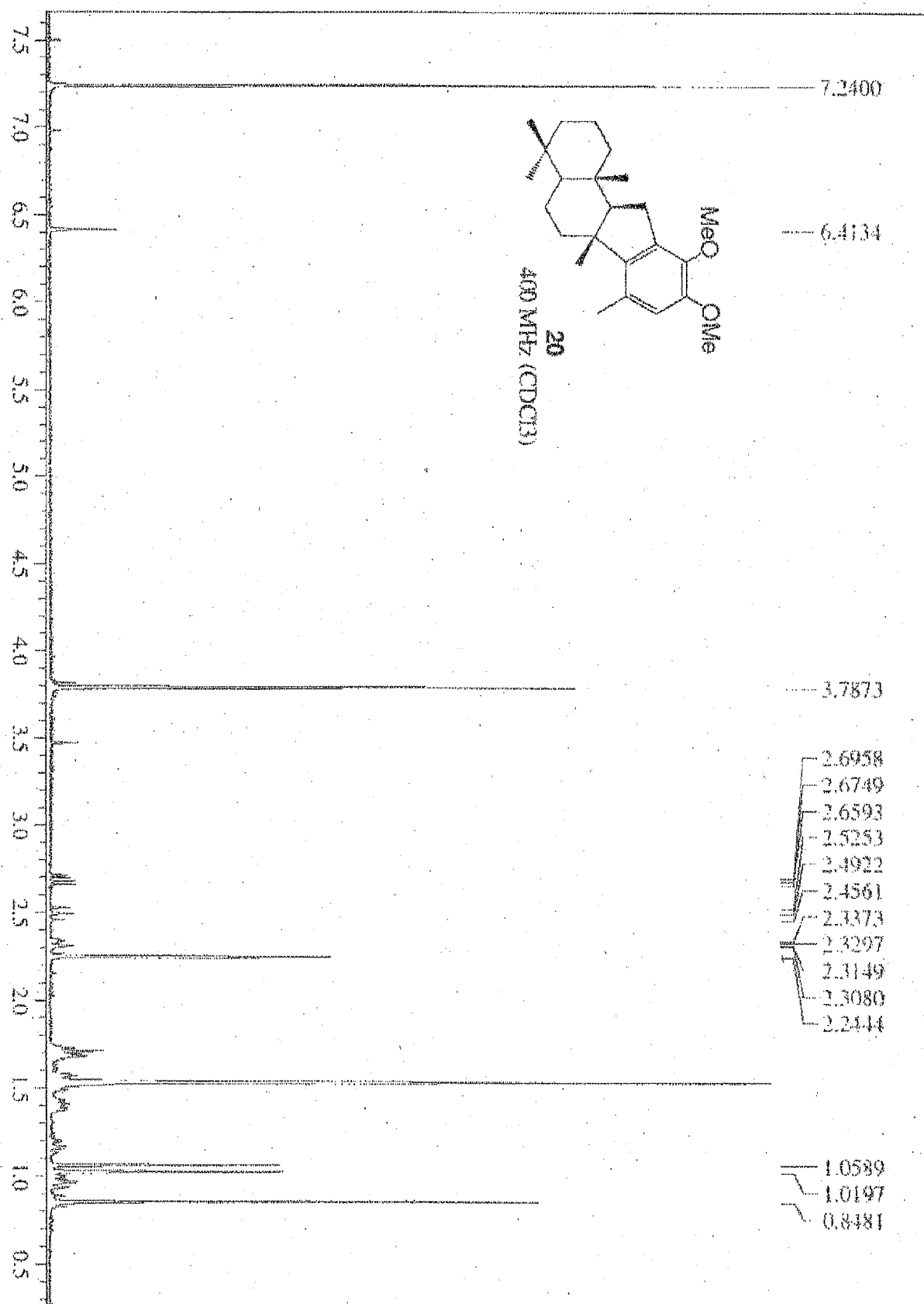


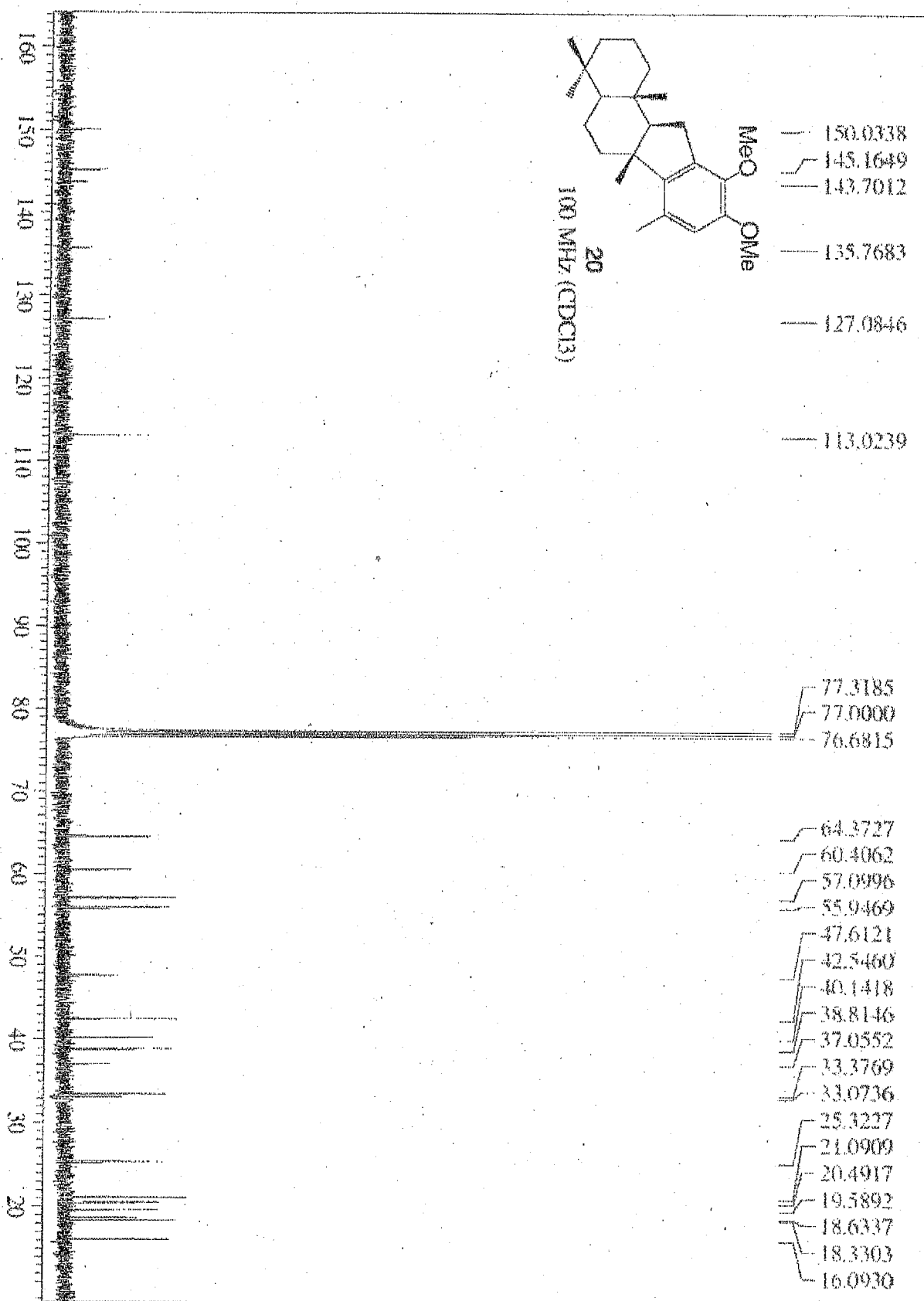


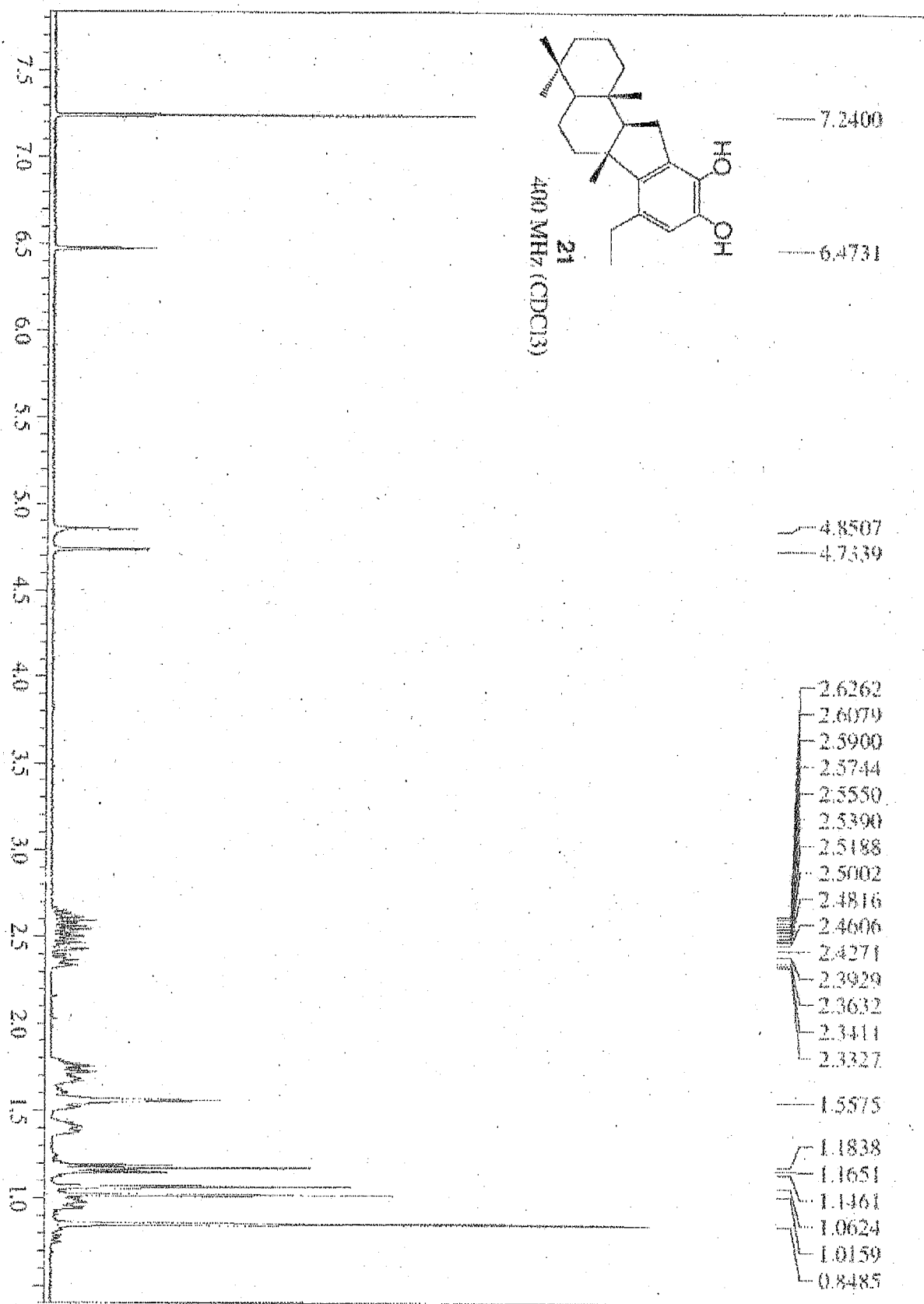


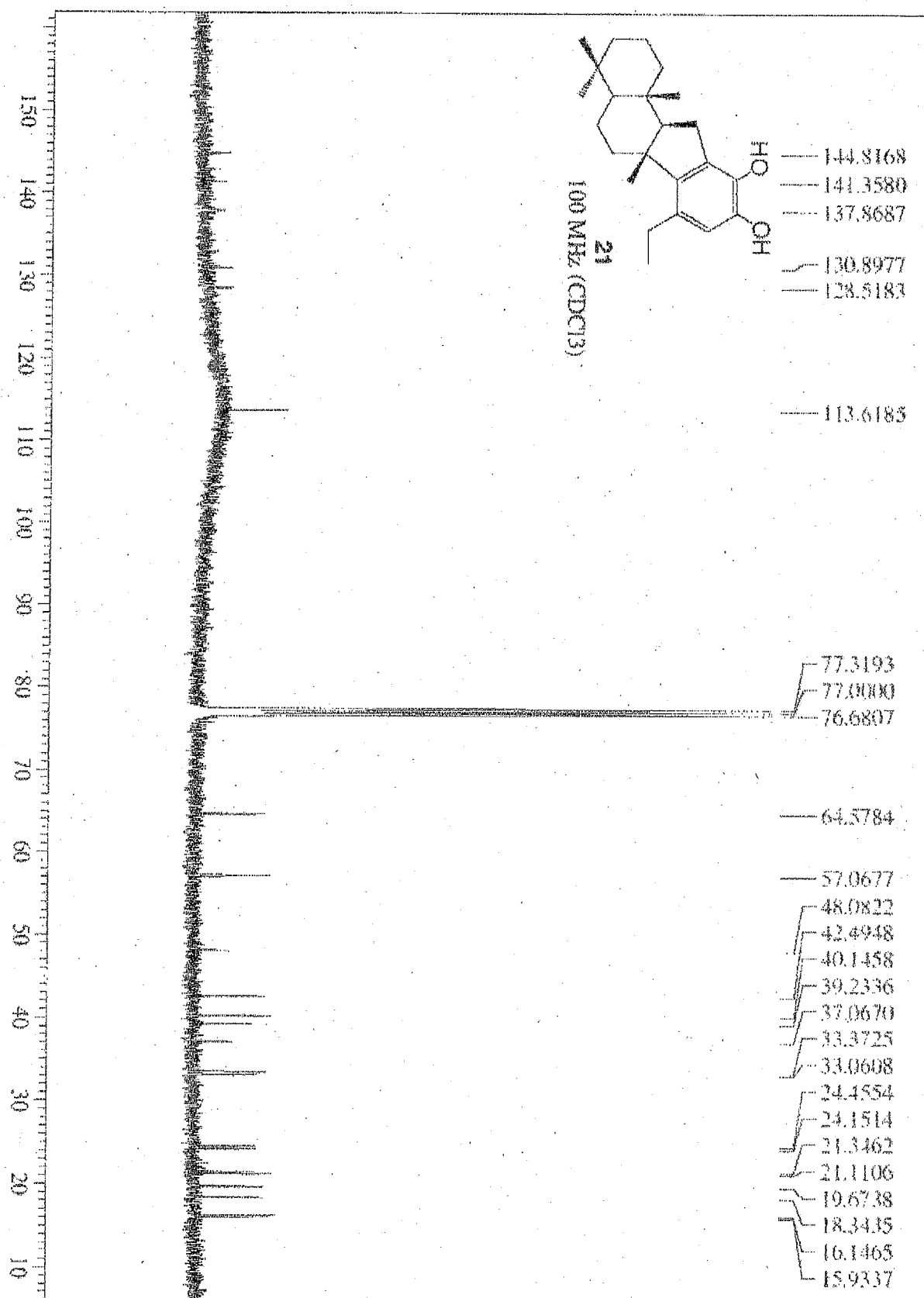


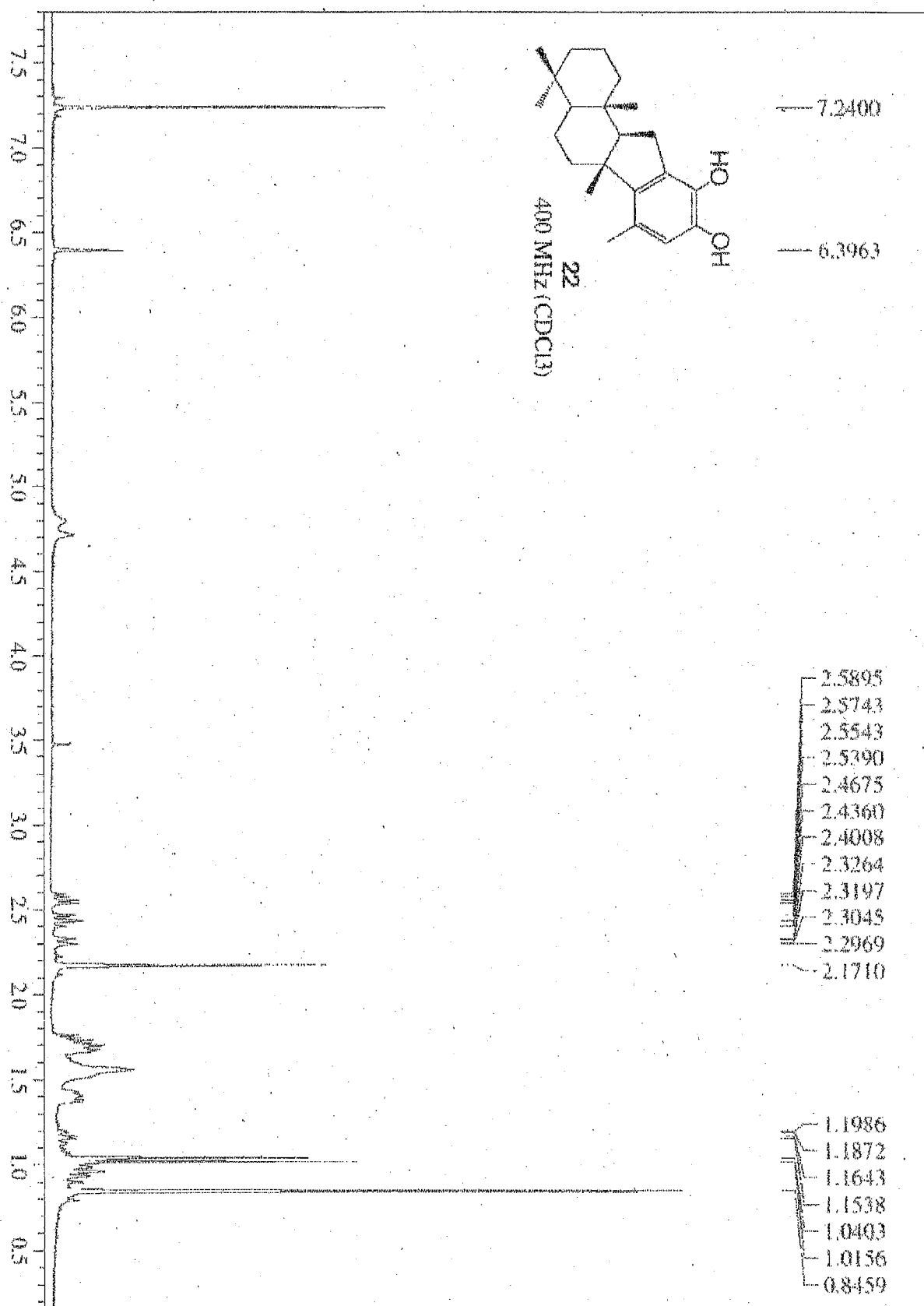


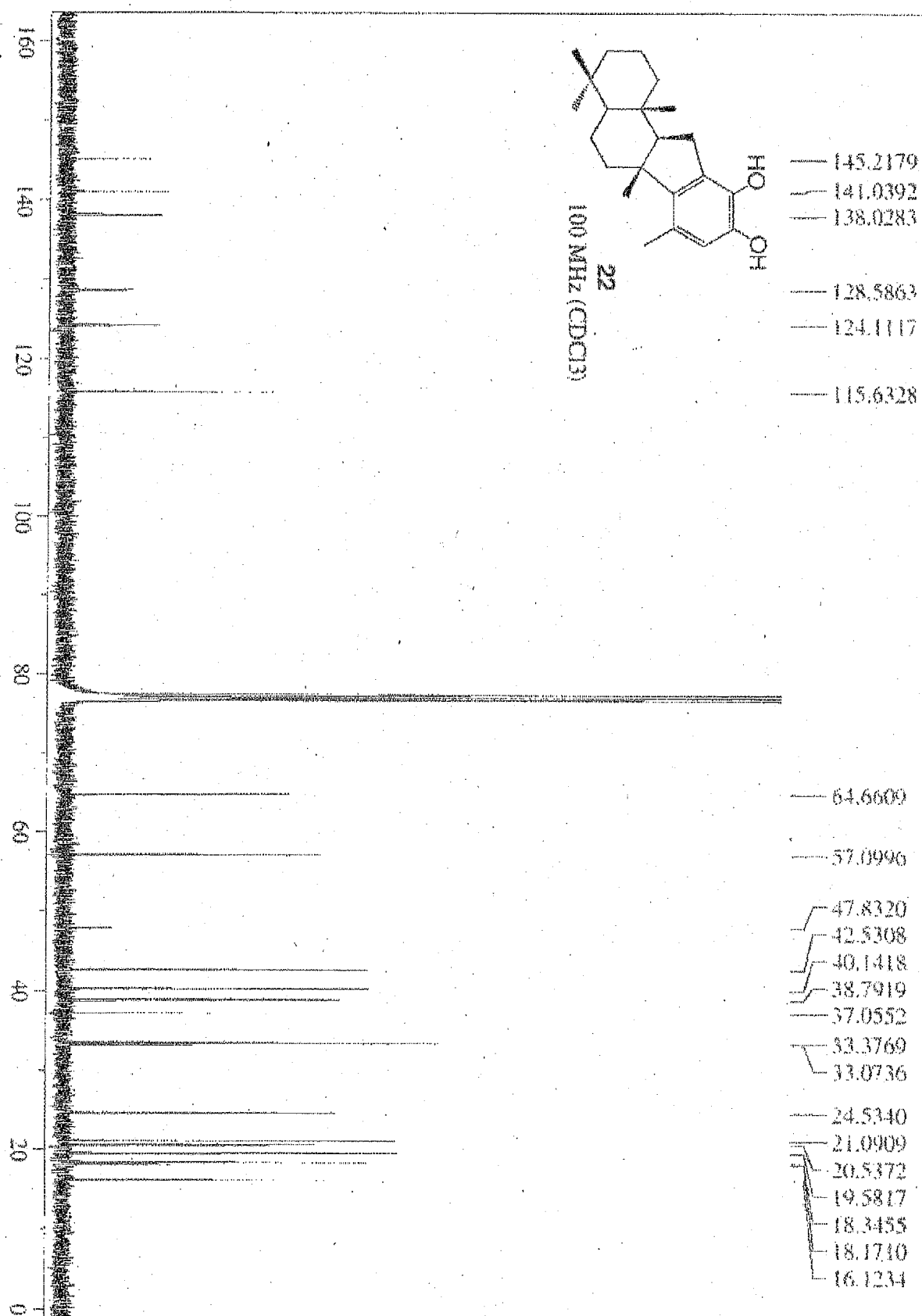


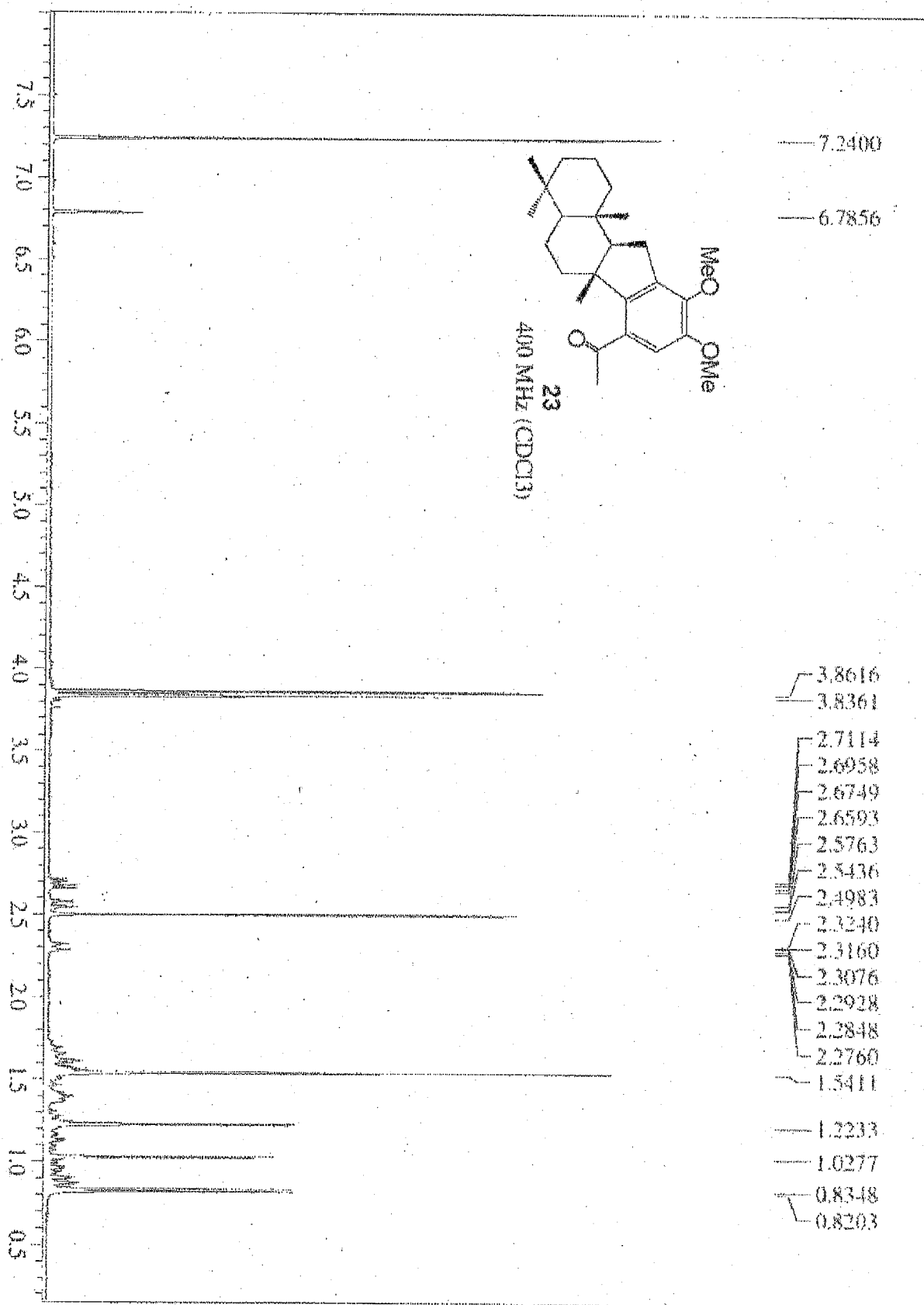


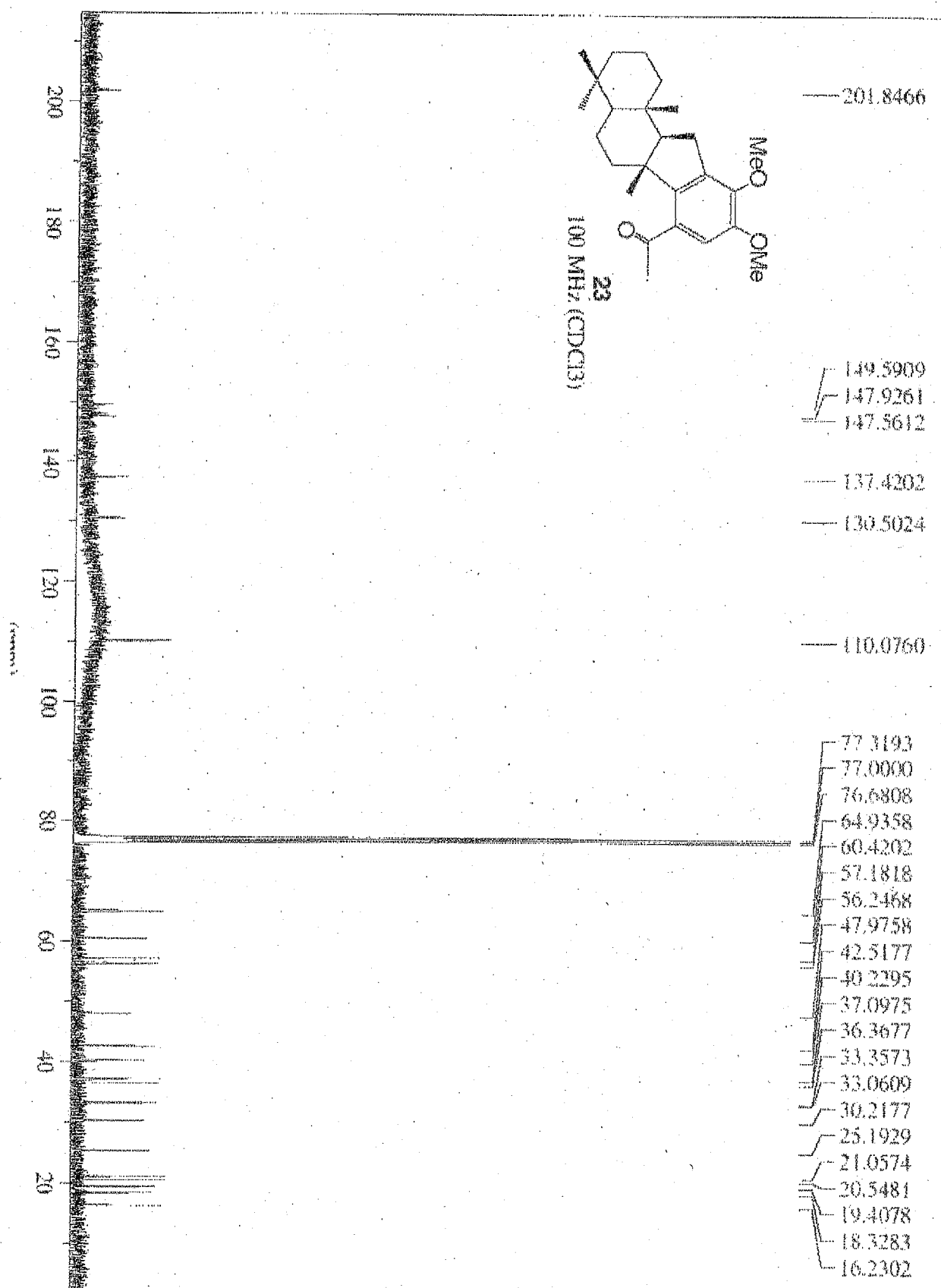


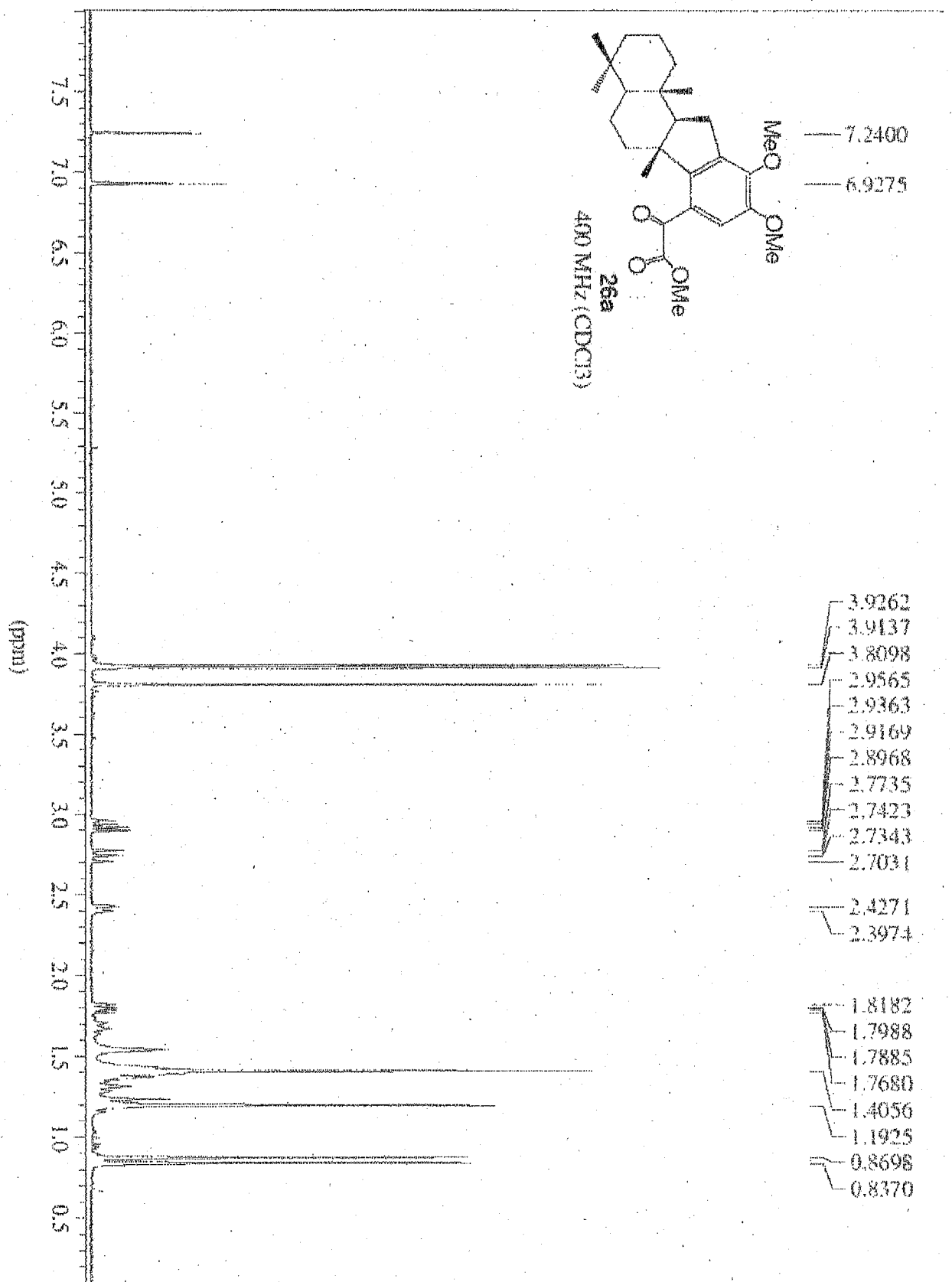


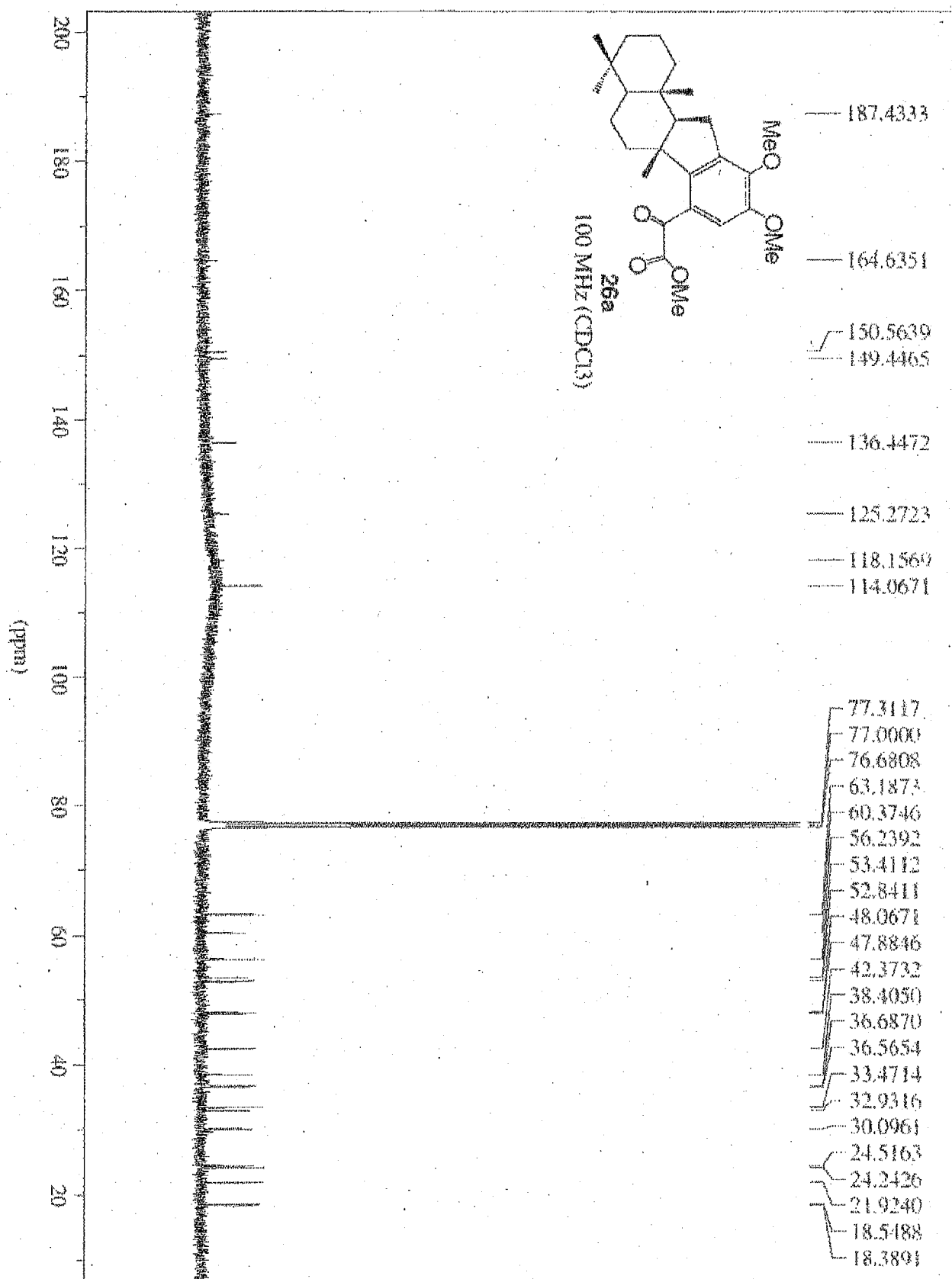


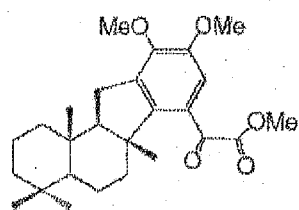




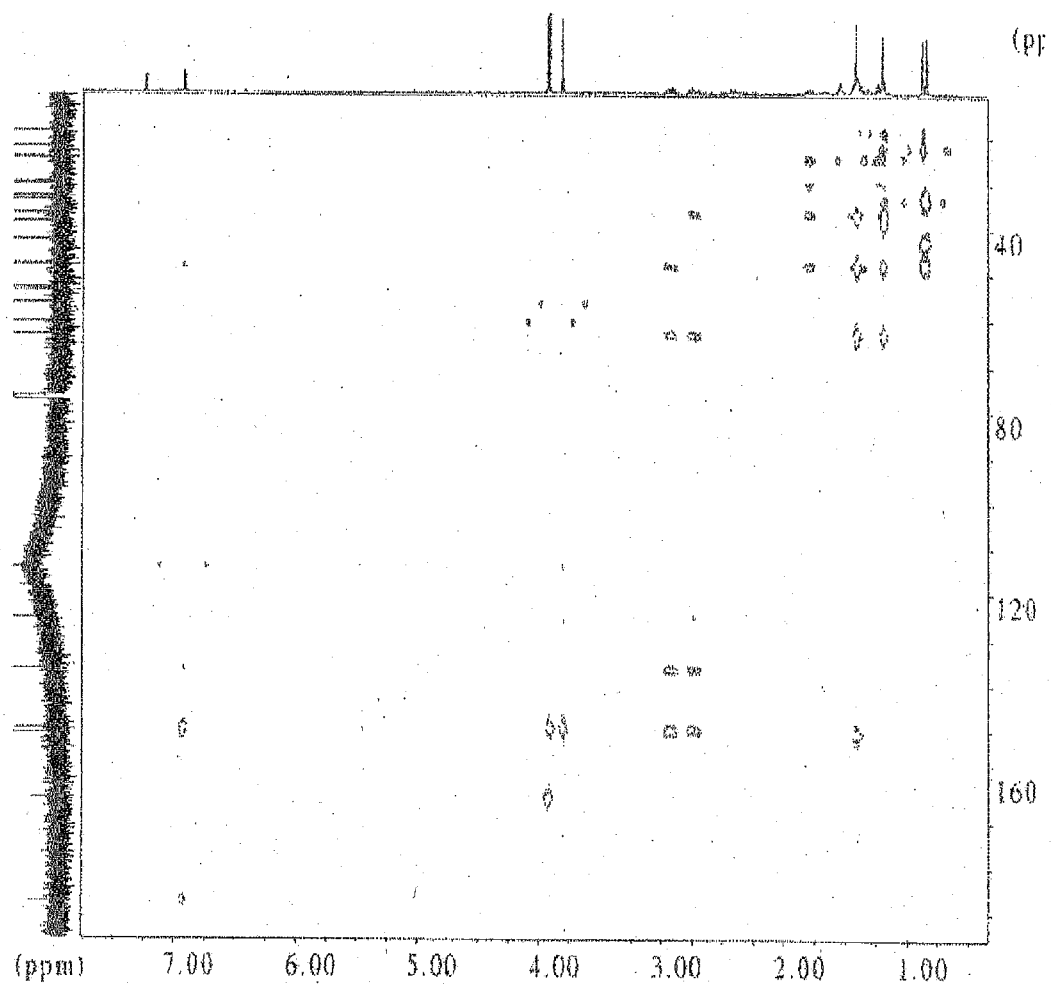


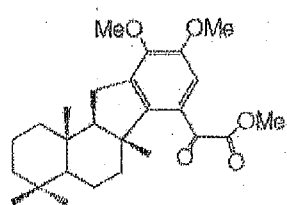




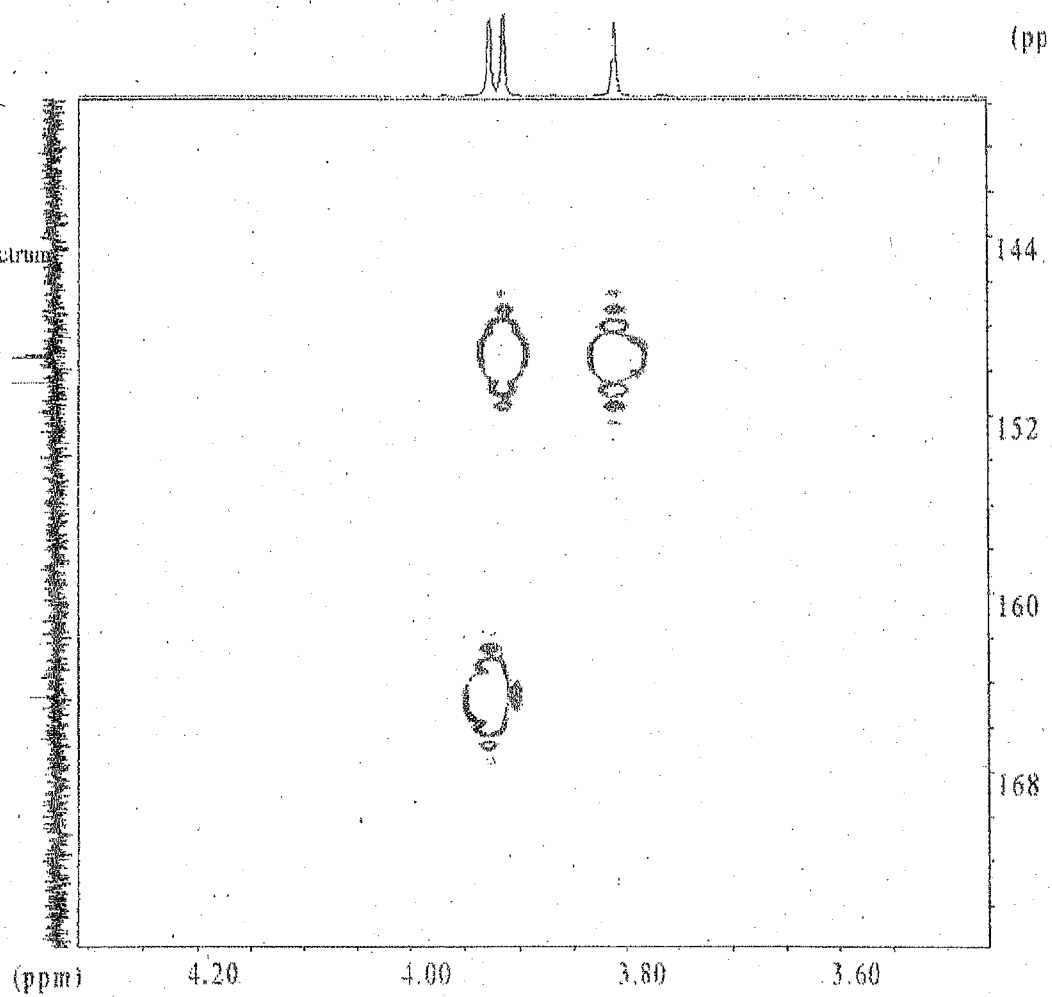


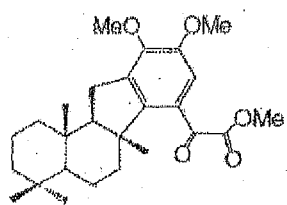
2D-HMBC spectrum of 26a  
in CDCl<sub>3</sub>



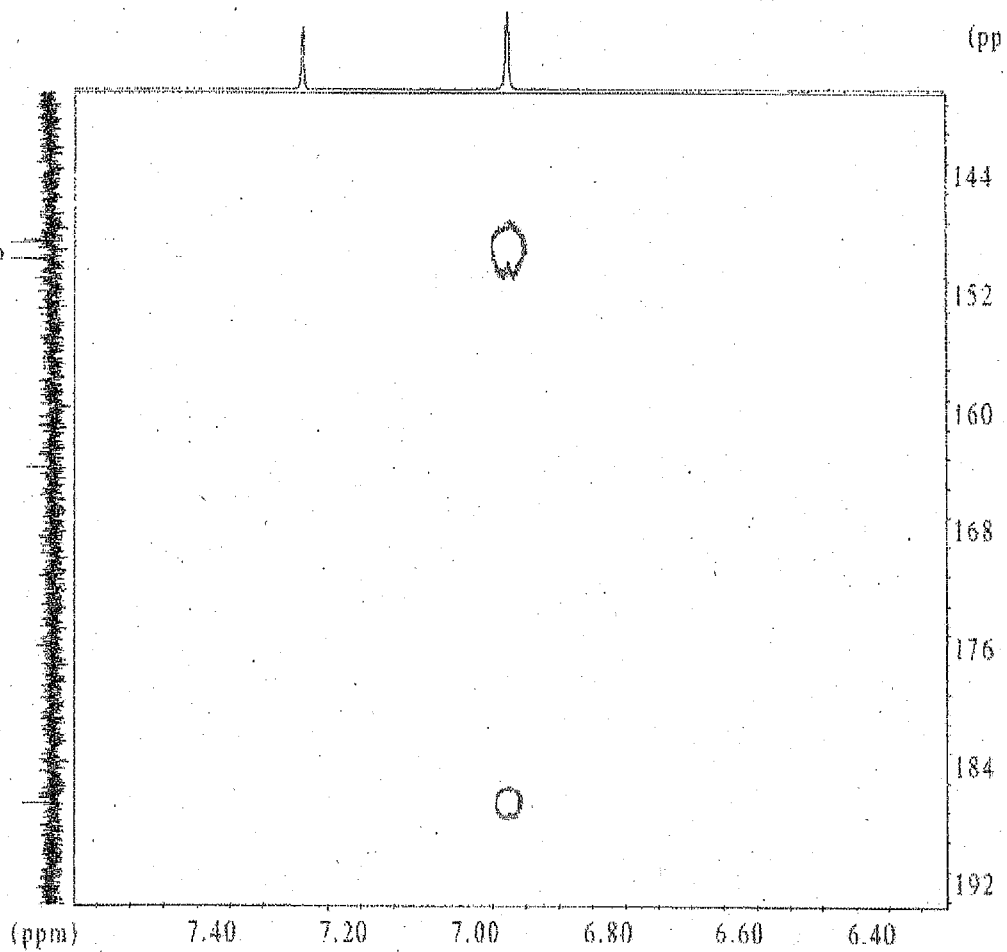


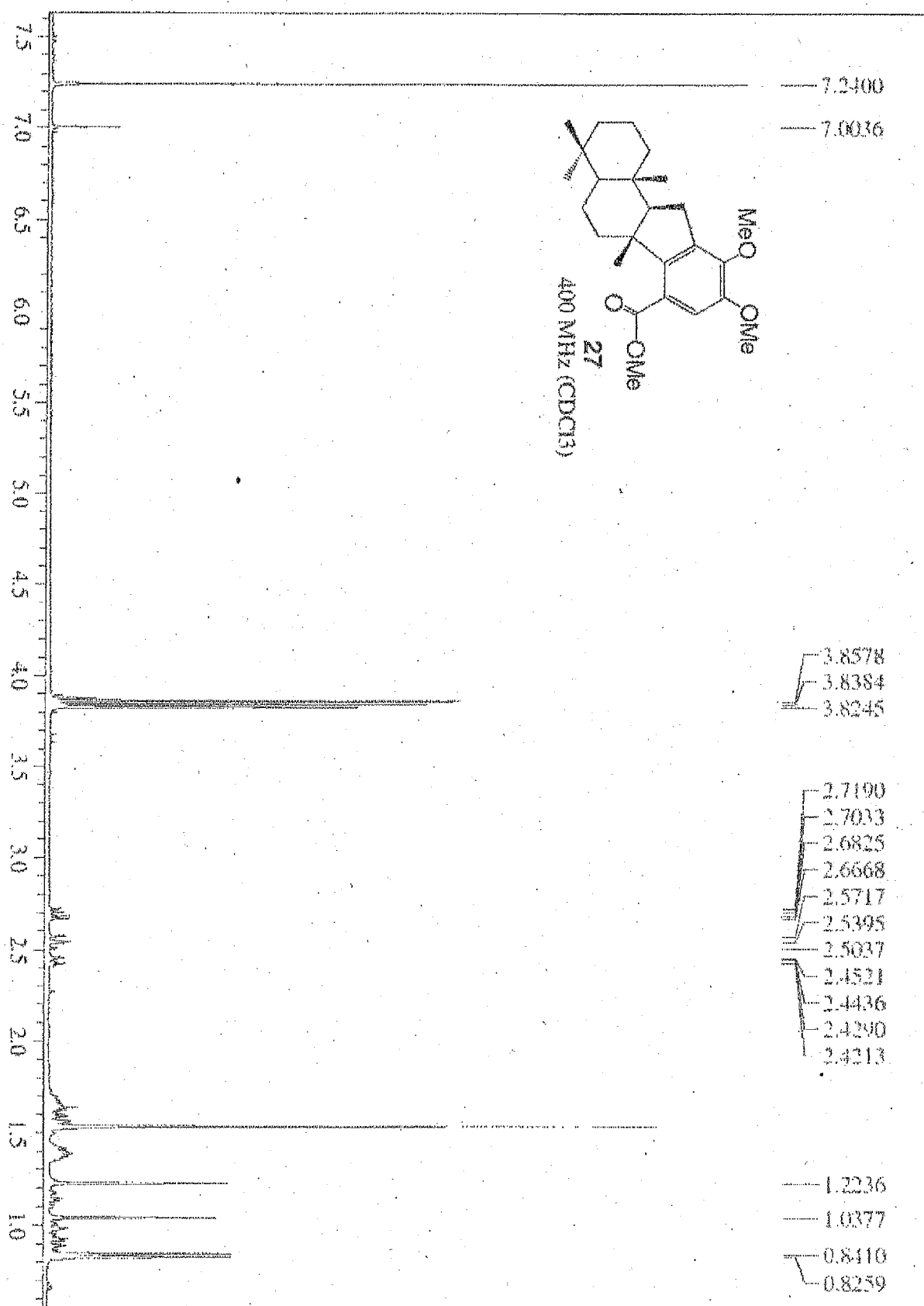
Selected region of 2D-HMBC spectrum  
of **26a** in CDCl<sub>3</sub>

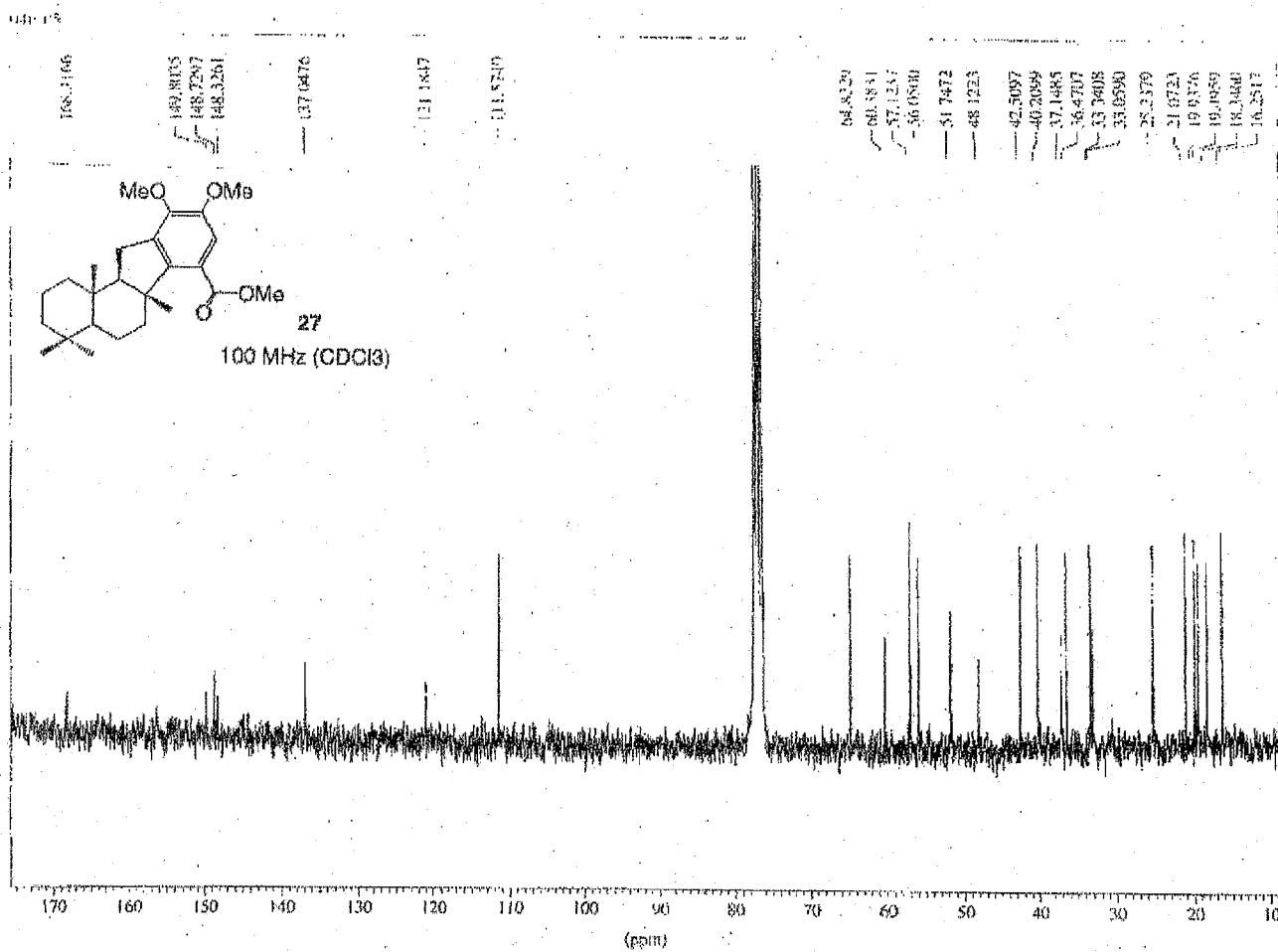


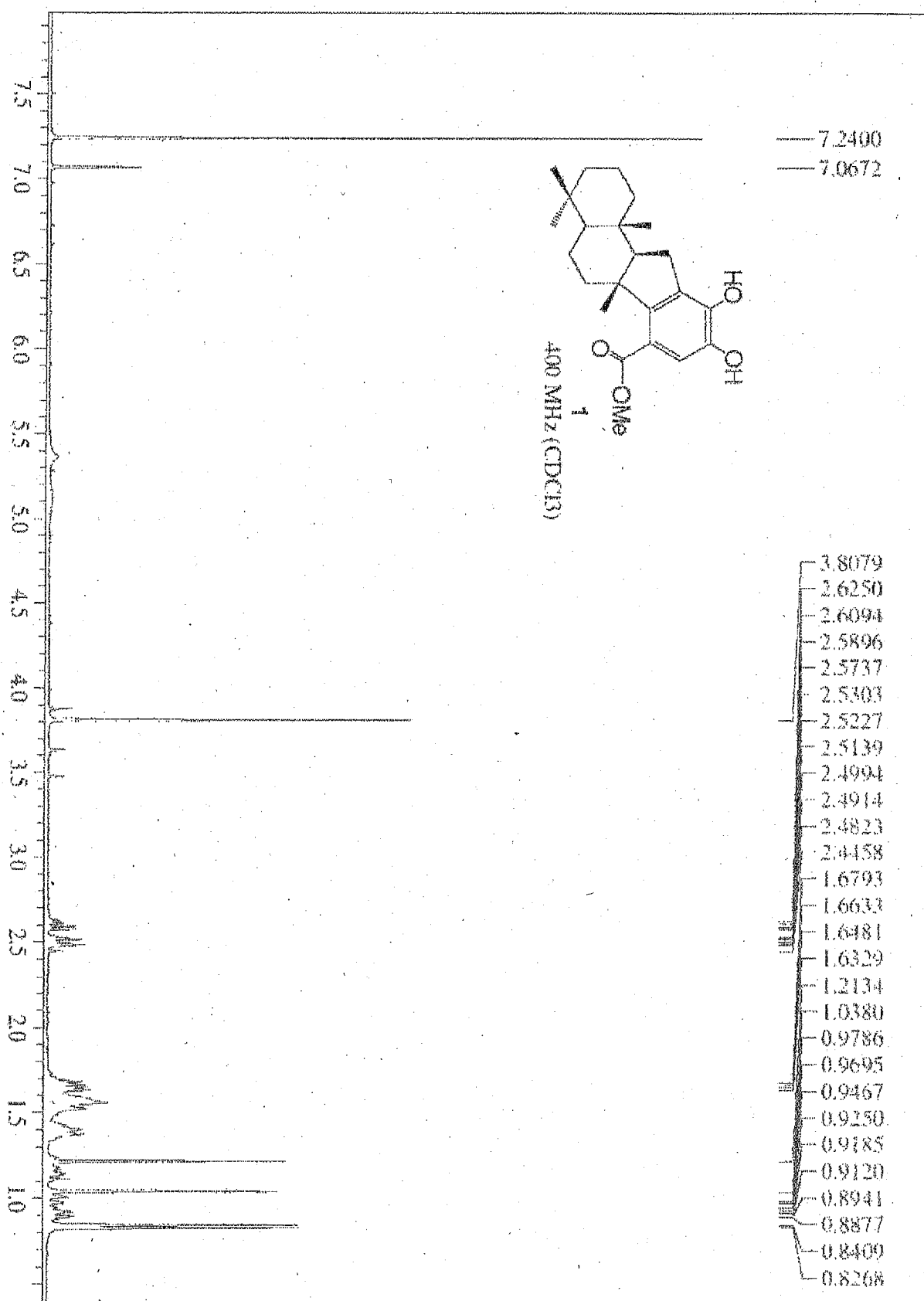


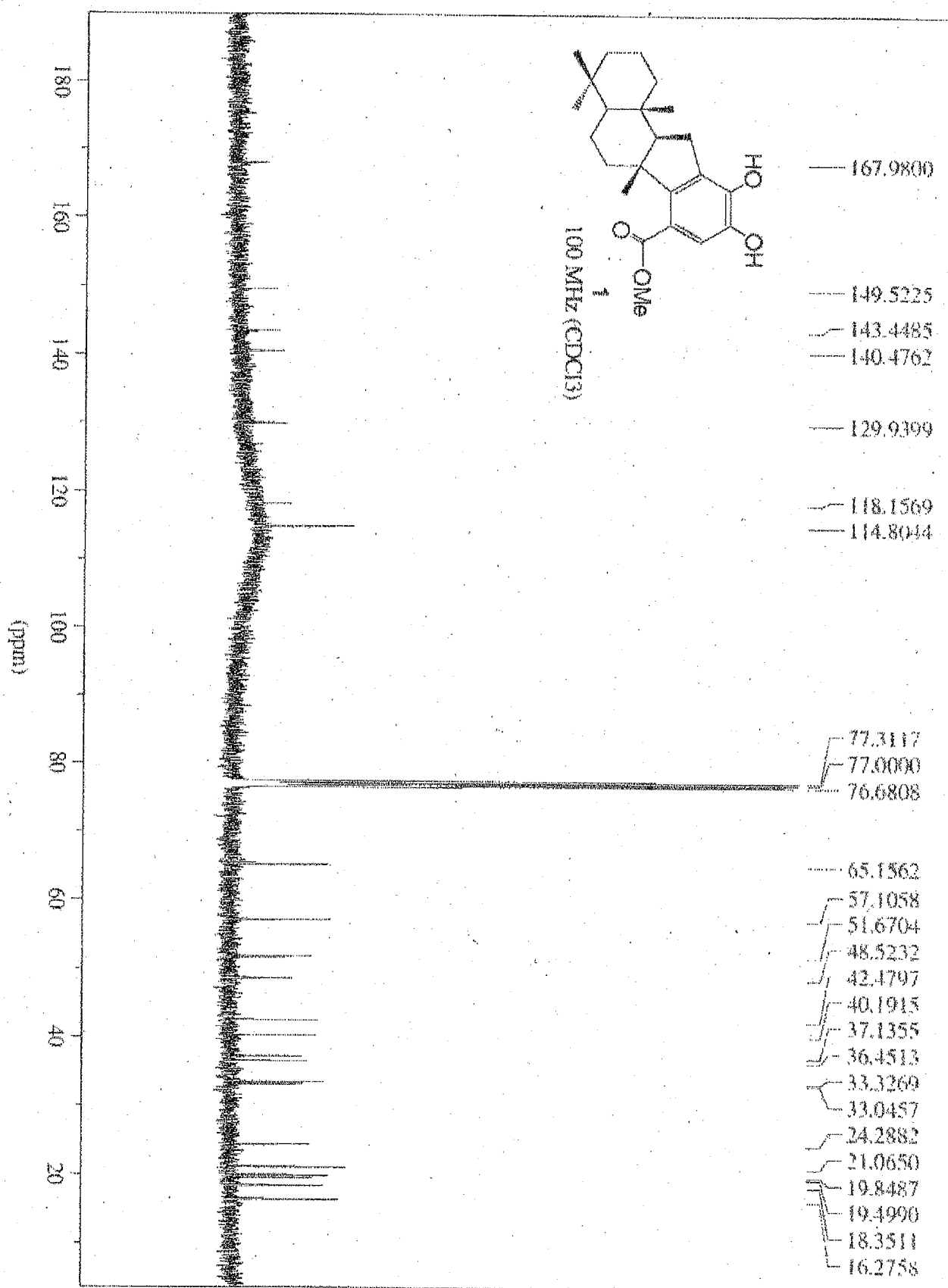
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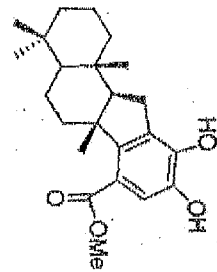












Pelanol 1  
isolated from  
*Dactyospongia elegans*

