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Chemical Constituents of *Plumbago indica* roots and reactions of plumbagin : Part II

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From *Plumbago indica* a new quinone derivative-plumbagic acid lactone, two flavonol methyl ethers-azaleatin (5-O-methylquercetin), ayanin (3,7,4′, tri-O-methylquercetin), two aliphatics-palmitic acid, myricyl palmitate and one naphthoquinone-plumbagin have been isolated. The major constituent plumbagin has been subjected to Schmidt reaction, allylic bromination, oxymercuration and demercuration and Thiele-Winter addition. All the natural and reaction products have been characterized by spectroscopic studies.

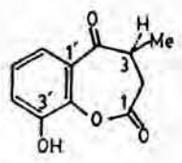
The roots of *Plumbago indica* Linn (syn. *P. rosea* L.) of family Plumbaginaceae, a shrub distributed in different parts of India is useful in folk medicine[1]. The isolation of several naphthoquinonoids and their derivatives, and flavonoids have been reported[2,3]. Our continued search for its chemical constituents has resulted in the isolation of plumbagin, palmitic acid and myricyl palmitate from the petrol extract and plumbagic acid lactone **1**, ayanin **7** and azaleatin **8** from the ethyl acetate extract of its roots. This is the first report of isolation of palmitic acid, myricyl palmitate, azaleatin and ayanin from *P. indica.* Isolation of ayanin **7** from *Alnus spp, Apuleia leiocarpa, Betula spp, Cheilanthes spp, Ostrya spp, Physalis angulata* and *Salvia glutinosa,* and azaleatin **8** from *Plumbago scandens* and *P. zeylanica* as glycosides have been reported[2,4]. The isolation of plumbagin has been reported earlier[5]. The major constituent plumbagin (5-hydroxy-2-methyl-α-naphthoquinone) has been reported to possess significant antitumor, antibacterial, antifungal and insecticidal activities[6-8]. In order to study the bioactivities of the reaction products, we carried out Schmidt reaction with sodium azide, allylic bromination with NBS, oxymercuration with mercury (II) acetate and demercuration with $NaBH_4$ and Thiele-Winter addition with Ac_2O. Herein, we report the isolation and characterization of both natural and reaction products on the basis of spectroscopic data.

Results and Discussion

The petrol extract of *P. indica* roots was separated into phenolic and neutral parts. The phenolic part on column chromatography (CC) over silica gel afforded palmitic acid **5**, mp 62°C (lit[9] mp 63°C) and plumbagin **2**, mp 78°C lit[10] mp 78°C). The neutral part on similar CC afforded myricyl palmitate **6**, mp 72°C (lit[9] mp 72°C).

The phenolic part of the ethyl acetate extract of *P. indica* roots on CC with Sephadex LH-20 followed by silica gel yielded plumbagic acid lactone **1**, mp 108°C; ayanin **7**, mp 172-74°C (lit[11] mp 172-74°C) and azaleatin **8**, mp 287°C. Identification of ayanin **7** was done by comparison of its spectral data with literature data[12], while of plumbagin **2** and azaleatin **8** by direct comparison with authentic samples. Palmitic acid **5** was identified from its spectral data as well as from comparison of the retention time of its methyl ester with that of authentic sample in gas chromatography. Identification of myricyl palmitate **6** was done by study of its spectral data as well as by characterization of spectral data of its saponification products palmitic acid and myricyl alcohol, mp 86°C (lit[9] mp 88°C).

Plumbagic acid lactone, $C_{11}H_{10}O_4$ (M^+206) showed UV-Vis spectrum in MeOH at λ_{max} 218 (logε, 4.17), 268 (4.02), 352(3.37) and 464nm (3.64) similar to that of plumbagic acid **9**[3]. It showed IR absorption bands in KBr at 3268 (OH), 1742 (COOAr) and 1700 cm^{-1} (>C=O). The ^1H NMR spectrum ($CDCl_3$) displayed signals for one methyl (δ 1.28, 3H, d, *J*=8 Hz, Me-3), three methines (δ 2.48, 1H, dd, *J*=18,6 Hz, H_α-2; 3.00, 1H, dd, *J*=18, 8Hz, H_β-2;3.88, 1H, br sextet, *J*=8Hz, H-3), three aromatic protons (δ 6.82, 1H, t, *J*=8 Hz, H-5′; 7.14, 1H, dd, *J*=8, 2Hz, H-4′;7.36, 1H, dd, *J*=8,



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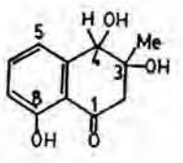

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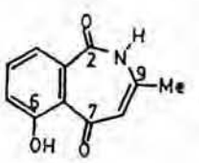

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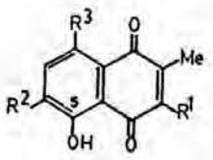
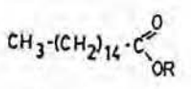
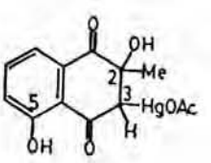


2: $R^1=R^2=R^3=H$
11: $R^1=Br$, $R^2=R^3=H$
12: $R^1=H$, $R^2=R^3=Br$



5: R=H
6: $R=CH_3-(CH_2)_{29}-$



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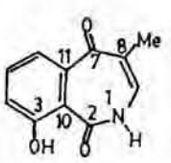

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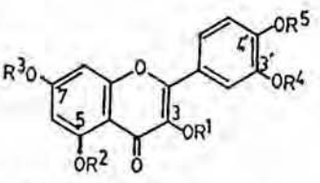
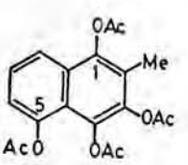


7: $R^2=R^4=H$, $R^1=R^3=R^5=Me$
8: $R^1=R^3=R^4=R^5=H$, $R^2=Me$



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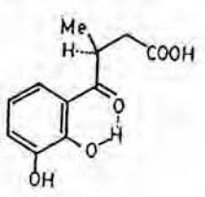
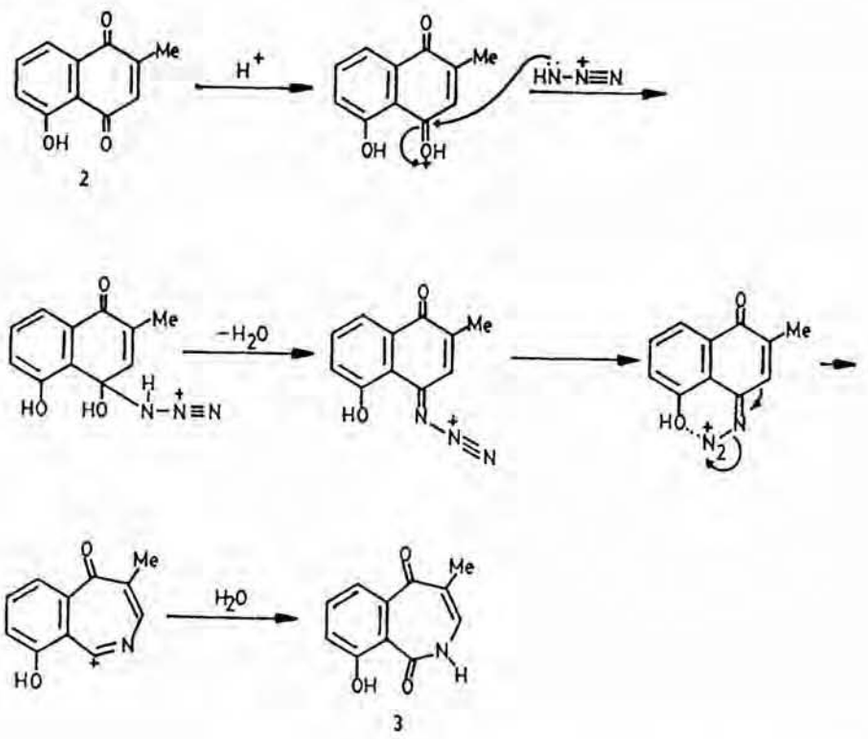


9

2 Hz, H-6′) and one chelated phenolic hydroxyl (δ 12.40, 1H, s, exchangeable with D_2O, HO-2) suggesting structure **1** for the compound. The FAB (positive) mass spectrum showed significant mass peaks at m/z 207$[M+H]^+$ (47%), 137 (100) and 121(20) also suggesting the said structure. The compound on hydrolysis with aq. methanolic H_2SO_4 gave plumbagic acid **9**, mp 110°C (lit[3] mp 112°C). The configuration at C-3 position was assigned as *S* by analogy with **9** of known absolute configuration. Possibly plumbagic acid was formed in plant by hydrolysis of **1**.

Study of Schmidt reaction on alkyl substituted benzoquinones with N_3H in conc H_2SO_4 at 0°C reported azepindiones as products[13]. We studied this reaction with plumbagin **2** using equimolar amount of NaN_3 under identical condition and found two isomeric products-compound A (yield 60%), mp 235°C and compound B(10%), mp 172°C. Compound A was characterized as 3-hydroxy-8-methyl-2, 7*H*-benzazepin-2,7-dione **3** and compound B as 6-hydroxy-9-methyl-2, 7*H*-benzazepin-2, 7-dione **10**, on the basis of spectral data (Experimental Section). The formation of the products can be rationalised on the basis of migratory aptitude of the groups towards electron deficient nitrogen. For unsymmetrical quinones, a smaller group of large orbital coefficient on carbon atom migrates preferentially because steric control plays major role over electronic effect in the yield of products[14-16]. Therefore, ethylene group migrates preferentially over phenyl group. The high yield of compound A was possibly due to migration of unsubstituted ethylene carbon and stabilization of the intermediate azide by intramolecular chelation. The possible mechanism of the reaction is outlined **(Scheme I).**



Scheme I

NBS is selective reagent for allylic bromination. We studied its scope in the bromination of **2**-alkylnaphthoquinone plumbagin **2** in CCl_4 in presence of a few drops of H_2O_2 and found two products characterized as 3-bromoplumbagin **11** (yield 70%), mp 128°C (lit[17] mp 120°C) and 6,8-dibromoplumbagin **12** (20%), mp 108°C. No allylic brominated product was found. This was possibly due to enone nature of quinone, which made the preferential attack of bromine at C-3 position. The phenolic hydroxyl group also activated the C-6 and C-8 positions of aromatic ring for electrophilic bromine addition.

Oxymercuration and demercuration of plumbagin **2** were not studied earlier. Hence, we carried out this reaction sequence by refluxing with $Hg(OAc)_2$ in aq. THF followed by reduction of the resultant acetoxy mercuri derivative **13** with alkaline $NaBH_4$ and found a tetralone **4** (yield 35%), mp 120°C. The absolute configuration of the tetralone **4** has not yet been established.

Both Asano *et al.*[18] and Cooke *et al.*[19] reported 1,3,4,5-tetraacetoxy-2-methylnaphthalene **14** as Thiele-Winter addition product of plumbagin **2** but no spectral data of the product was reported. We reinvestigated the reaction with Ac_2O and a drop of conc H_2SO_4 or fused NaOAc. In both cases we found the same product **14**, mp 180°C (lit[19] mp 176.5°C) along with another product, mp 270°C (lit[19] mp 283°C). The latter product was not characterized because of its insolubility in common organic solvents. It was observed that the yield of **14** was higher with NaOAc (50%). The spectral data of **14** is reported (Experimental Section). Biological testings of the reaction products are in process.

Experimental Section

Mps were measured on a Kofler block. UV-VIS spectra were recorded on a Spectronic 21 spectrometer, IR spectra on Perkin Elmer 577 spectrometer, ^1H NMR and ^{13}C NMR spectra on Jeol FX-100 and Bruker WM 400 spectrometers using TMS as internal reference, GC on Pye Unicam 104 gas chromatograph and MS on a Shimadzu QP-2000 spectrometer. Carbon chemical shifts were assigned by DEPT/APT studies. Column chromatography (CC) and thin-layer chromatography (TLC) were performed using silica gel (Mesh 60-120, Qualigens) and silica gel G (Merck), respectively, *Plumbago indica* Linn roots were collected from Hooghly in December 1994.

Extraction of *P. indica* roots and isolation of compounds

The air-dried powdered roots (2 kg) of *P. indica* was extracted successively with petrol (bp 60-80°C) and EtOAc in a Soxhlet type extractor. Each extract after removal of solvent under reduced pressure was separated into phenolic and neutral parts by treatment with 5% NaOH followed by acidification of the aq. Alkaline part with 2*M* HCl and extraction with Et_2O.

The phenolic part (6.2g) of the petrol extract was column chromatographed. The early fraction of petrol $-C_6H_6$ (6:4) eluate gave a residue which on repeated CC afforded palmitic acid in colourless granules (0.04 g, 2×10^{-3}%), mp 62°C; IR (KBr): 1704 cm^{-1} (CO_2H); ^1H NMR ($CDCl_3$) : δ 0.86 (3H,t,Me); 1.24(26H, br, s, $CH_2\times13$), and 2.32 (2H, t, *J* =6Hz,CH_2 adjacent to CO_2H); MS (EI) : m/z 257 $[M+H]^+$ (51%), 242(6), 228(11), 214(30), 200(6), 186(26), 172(21), 158(14), 144(7), 130(57), 73(100) and 60(85); GC of methyl ester : PEGA column; inj. Port, column and FID temperatures were 200°C, 190°C and 210°C, respectively. The petrol-C_6H_6 (1:1) eluate gave plumbagin **2** (3.6g, 18×10^{-2}%) recrystallised from petrol, mp 78°C.

The neutral part (1.5g) of the petrol extract on CC gave myricyl palmitate **6** from petrol $-C_6H_6$ (7:3) eluate in granular crystals, mp 72°C (0.06g, 3×10^{-2}%); IR(KBr): 1740 cm^{-1} (ester); ^1H NMR($CDCl_3$): δ 0.84 (3H, distorted t, Me) 0.88 (3H, distorted t, Me), 1.26 (80H,brs, CH_2x40), 1.54(2H,brs, CH_2adjacent to OCH_2), 2.30(2H,t,CH_2 adjacent to - COO) and 4.07 (2H,t,OCH_2-); MS (EI) : m/z 676$[M]^+$ (4%), 648(7), 620(8),592(7), 564(5), , 285(11), 257(100), 256(30), 239(9), 111(32), 97(47), 83(64), 71(75), 57(91) and 43(50); saponification with 1*N* methanolic KOH under N_2 atmosphere gave myricyl alcohol (1-triacontanol), $CH_3(CH_2)_{29}$ OH, mp 86°C and palmitic acid, mp 63°C.

The phenolic part of the EtOAc on CC with Sephadex LH-20 using MeOH-EtOAc -Me_2CO (1:3:6) afforded two fractions. The 1st fraction on repeated CC with petrol - EtOAc. (4:1) mixture gave plumbagic acid lactone (1) in colourless needles (0.08g, 4×10^{-3}%), mp 108°C (from petrol - EtOAc) [Found : C, 63.95; H,4.78. Calc for $C_{11}H_{10}O_4$: C, 64.07;H,4.88%]. The 2nd fraction on repeated CC using petrol-EtOAc (3:2) mixture afforded ayanin **7** in colourless needles (0.05g, 2.5×10^{-3}%), mp 172-74°C and azaleatin **8** in pale yellow needles (0.04g, 2×10^{-3}%), mp 287°C.

Plumbagic acid lactone 1 : ^{13}C N MR($CDCl_3$) : d 18.44 (Me-3), 36.57(C-2), 37.00(C-3), 117.92 (C-1'), 119.14(C-4'), 120.44 (C-6'), 120, 61 (C-5'), 145.00 (C-3'), 150.32(C-2'), 175.80(C-1), 208.50(C-4).

Ayanin 7: UV-Vis (MeOH): 256(loge, 3.94), 370 (3.88) and 452 nm. (3.90); (MeOH+ OH^-): 68, 422 and 524 nm; (MeOH+$AlCl_3$) : 268 and 428 nm; (MeOH +H_3BO_3): 212, 256 and 370 nm; IR (KBr): 3318(OH), 1654(>C=O) and 1597 cm^{-1} (>C=C<); ^1H NMR ($CDCl_3$) : d 3.92(3H,s,MeO-4'), 4.02 (6H,s, MeO-3,7), 6.38(1H,d,*J*=2 Hz,H-2), 6.54 (1H,d,*J*=2 Hz, H-8), 6.04 (IH, s,exchangeable with D_2O, HO-3'), 7.02(IH,d,*J*=9 Hz, H-5'), 7.78(IH,d,*J*=2, Hz,H-2'), 7.84(IH,dd,*J*=9,2Hz,H-6'), and 11.76(IH,s, exchangeable with D_2O, HO-5) ; MS(EI): m/z 344$[M]^+$ (100%), 343$[M-1]^+$ (8), 329 $[M-Me]^+$ (21), 314$[M-30]^+$ (5), 313(4), 301$[M-43]^+$(27), 286(5), 283(6), 273(7), 258(8), 230(5), 167(5) and 151(4).

Azaleatin 8 : UV-Vis : (MeOH): 254(loge,4.05) and 370nm(3.95); (MeOH+OH^-): 240, 285, 330 and 432nm; (MeOH + $AlCl_3$): 272 and 450 nm; ^1H NMR (DMSO-d_6) : d 3.98. (3H, s,MeO-5),. 6.20(1H, d, *J*=2 Hz,H-6), 6.04 (1H,d,*J*=2 Hz,H-8), 6.84 (1H,d,*J*=9 Hz,H-5'), 7.52(IH, d,*J*=2 Hz, H-2') and 7.68(IH, dd, *J*=9,2 Hz,H-6'); MS (El) : m/z 316$[M]^+$ (100%), 315 $[M-1]^+$ (63), 299$(M-OH]^+$ (5), 298$[M-H_2O]^+$ (30), 287 (23), 271(22), 167(14), 166(8), 137(18) and 109(16).

Hydrolysis of plumbagic acid lactone 1 : An aqueous- methanolic solution (10mL) of 1(0.03g) was refluxed with 2*N* H_2SO_4 for 2 hr and usual work up of the reaction mixture afforded plumbagic acid **9** (0.01g), mp 110°C.

Schmidt reaction of plumbagin 2: A solution of **2** (0.5g) in conc H_2SO_4 (10mL) was cooled at 0°C in ice bath and NaN_3 (0.2g) was slowly added. The solution was kept as such for 1 hr when evolution of N_2 ceased. The solution was then poured in a mixture of ice and water, when a precipitate appeared. It was filtered and the filtrate was extracted with EtOAc. Both the precipitate and the residue of EtOAc extract were mixed together and subjected to column purification when compound A(3) was obtained in orange needles (0.3g), mp 235°C and compound B(10) in blackish- violet needles (0,05g), mp 172°C.

Compound A(3): UV-Vis (MeOH) : 224 (loge, 4.27), 308 (4.03) and 478nm (3.71); IR (KBr) : 3180 (OH, NH), 1678 (lactam >C=O) and 1640 cm^{-1} (unsaturated keto >C=O); ^1H NMR ($CHCl_3$): δ 2.30 (3H, s, Me-8), 6.48(1H, dd, *J*=8, Hz, H-4), 6.78(1H,

dd, *J*=8,2 Hz, H-6), 6.98 (1H brs,H-9), 7.45(1H,t, *J*=8 Hz,H-5) 8.64 (1H,brs, exchangeable with D_2O, HN-1), and 13.40(1H, s, exchangeable with D_2O,HO-3); ^{13}C NMR ($CDCl_3$+DMSO d_6) : δ 21.53 (Me), 110.07 (C-4), 111.22 (C-6), 111.62 (C-11), 136,21 (C-9), 137.16 (C-5), 139.73 (C-10), 142.90 (C-8), 162.92 (C-3), 163.15 (C-2), and 190.59 (C-7); MS (EI) : m/z 203$[M]^+$ (86%) (Calc for $C_{11}H_9NO_3$, 203.197), 175$[M-CO]^+$(52), 167 (18), 149 (52), 147 $[175-CO]^+$ (32), 142(60), 132(5), 118(16), 92(22), 57(86) and 43(100).

Compound B(10): UV-Vis (MeOH): 256 (loge, 4.20), 302 (3.96) and 408 nm (3.65); ^1H NMR ($CHCl_3$): δ 2.64 (3H, s, Me-9), 6.48 (1H, dd, *J*= 8,2 Hz, H-5), 6.84 (1H, dd, *J*=8, 2 Hz, H-3), 7,04(1H, brs, H-8), 7.48 (1H, t, *J*=8 Hz, H-4), 8.54 (1H, brs,. exchangeable with D_2O, HN-1), and 13.40 (1H, s, exchangeable with D_2O, HO-6); MS(EI): m/z 203$[M]^+$(100%), 175(31), 148(29), 147(14), 146(11), 132 (13), 118(16) and 92(26).

NBS bromination of plumbagin 2: A solution of 2(0.5g) in CCl_4 (15mL) containing 5 drops of H_2O_2 was refluxed with NBS (0.8g) for 10 hr. The reaction mixture was filtered and the filtrate was concentrated and purified by CC when 3-bromoplumbagin **11** was obtained in orange needles (0.35g) mp 128°C and 6.8-dibromoplumbagin **12** in red needles (0.1g), mp 108°C. 3-Bromoplumbagin **11** was identified in mixed mp and co-TLC with an authentic sample available in the laboratory.

6,8- dibromoplumbagin (12): UV-Vis (MeOH) : 214 (loge, 4.55), 288 (4.24) and 4.52 nm (4.07) ; 1R(KBr): 1665 (unchelated > C=O) and 1635 cm^{-1} (chelated > C=O); ^1H NMR ($CDCl_3$) : δ 2.20 (3H,d,*J*=1.5 Hz, Me-2), 6.82 (1H,q, *J*=1.5 Hz, H-3), 7.62 (1H,s, H-7), and 12.97 (1H, s, exchangeable with D_2O, HO-5); MS (EI) : m/z 348$[M+4]^+$ (25%), 346 $[M+2]^+$ (41), 344[M](21), 333 (2), 331(4), 329 (2), 267 (94), 265(100), 252(2), 248(5), 239(6), 237$[265-CO]^+$ (8), 222 (11), 209(5), 186(72),171(6), 158 (65), 130(39), 120(13) and 90(20).

Oxymercuration of plumbagin 2: A solution of **2**(0.2g) in aqueous-THF (10mL) was refluxed with Hg $(OAc)_2$ (0.4g) for 4 hr. The reaction mixture was evaporated to a residue, diluted with water, and extracted with $CHCl_3$. The concentrated $CHCl_3$ extract on column purification afforded 2,3H-3-acetoxymercuri-2-hydroxyplumbagin **13** in orange needles (0.15g) mp 263°C. UV-Vis(EtOH) : 210 (loge, 4.57), 274(4.44) and 436nm (3.97); (EtOH +OH^-) : 212, 282 and 448nm ; IR(KBr); 3500(OH), 1730 (ester), 1665 and 1620 cm^{-1} (unchelated and chelated >C=O), ^1H NMR ($CDCl_3$): δ 1.54(3H,s, Me-2), 2.38(3H,s, MeCOOHg-3), 3.10(1H, s, H-3), 7.28-7.62 (3H, m, H-6,7,8) and 11.72(1H, s, exchangeable with D_2O, HO-5); MS(FAB-positive) : m/z 462 $[M+H]^+$ (12%), 205(8), 177(12), 149(22) and 120 (18), base peak at 154 due to matrix, *m*-nitrobenzyl alcohol.

Demercuration of acetoxymercuri plumbagin 13: To a solution of 13 (0.1g) in THF (10mL), an alkaline solution (10mL) of $NaBH_4$ (0.5*M* $NaBH_4$ in 3*M* NaOH) was added and the resultant solution was stirred for 1 hr and organic layer was separated out. It was dried , concentrated and purified by CC when orange crystals (0.O8g) of tetralone (4), mp 120°C was obtained. UV-Vis (EtOH): 228 (loge, 4.15), 282 (4.09) and 462nm (3.61); IR(KBr): 3300 (OH) and 1630 cm^{-1} (chelated >C=O); ^1H NMR ($CDCl_3$) : δ 1.60 (3H, s, Me-3), 2.10-2.30 (2H, m, H_2-2), 3. 65 (1H, s, H-4). 7. 20 (1H, dd, *J*=8, 2 Hz, H-7), 7.60 (1H, dd, *J*=8,2 Hz, H-5) 7.68 (1H, t, *J*=8 Hz, H-6), and 11.14 (1H,s,exchangeable with D_2O, HO-8); MS (EI): m/z 208$[M]^+$ (100%), 176$[M-MeOH]^+$ (21), 158(10), 148(21), 130 (16) and 120(7).

Thiele-Winter addition reaction of plumbagin 2: To a solution of **2** (0.02g) in Ac_2O (3mL), conc H_2SO_4 (2 drops) was added and the solution was kept for 3 days. The usual work up of the reaction mixture followed bycolumn purification gave 1,3,4,5- tetra-acetoxy-2-methylnaphthalene **14** in colourless needles (0.04g), mp 180°C from C_6H_6-EtOAc (4:1) eluate. The EtOAc eluate afforded another compound in colourless needles (0.03g), mp 270°C.

The reaction was repeated using NaOAc (0.2g) instead of conc H_2SO_4 and keeping the quantities of the other reactants same. The solution was kept overnight. The usual work up of the reaction mixture followed by column purification gave **14** (0.10g).

1,3,4,5,- Tetraacetoxy-2- methylnaphthalene 14 : UV(EtOH) : 236 (loge, 4.40), 300 (3.58) and 332nm (3.20); IR (KBr) : 1760 cm^{-1} (>C=O); ^1H NMR ($CDCl_3$): δ 2.16 (3H, s, MeCOO-5), 2.32 (6H, s, MeCOO-1,4), 2.40 (3H, s, MeCOO-3), 2.46 (3H, s, Me-2) and 7.48-7.80 (3H,m,H-6,7,8); MS (EI) : m/z 374 $[M]^+$ (32%), 188 (100), 160 (26), 132 (16), 120 (20), 92 (20) and 43 (84).

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