



Chemical constituents of montan resin from Yunnan Esan

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ABSTRACT

Large amount of montan resin is generated as by-product during production of refined montan wax from montan wax and cannot recycle. In order to elucidate material basis for realizing ecological utilization, fourteen compounds were isolated from the montan resin of Yunnan Esan (China) by modern chemical separation methods, and their structures were elucidated by spectroscopic methods. They were identified as 31-homohopanoic acid (**1**), bacteriohopanetetrol (**2**), stigmaterol (**3**), 24,25-dinoroleana-1,3,5-triene-19,28-epoxy (**4**), bis-(7-methyl-nonane) phthalate (**5**), terephthalic acid-cyclic ethylene ester (**6**), 3,4-quinoxaline (**7**), 2-hydroxy-carboxyl-phthalic acidooctyl ester (**8**), dacane cyclohexane (**9**), methyl dodecyl (**10**), methyl heptyal (**11**), hendecane (**12**), decane (**13**), octane (**14**). Those fourteen compounds were isolated from montan resin for the first time.

Key words: lignite; montan resin; chemical constituent; triterpene; NMR

INTRODUCTION

To produce humic acid and montan wax from lignite is environmentally friendly and efficient way to utilize lignite. Compared with the way to burning, more and more countries and companies are more inclined to produce high value-added and green products from lignite, such as humic acid and montan wax. As is known to all, humic acid, as a natural product, is used widely in forestry, agriculture, pharmacy, chemical industry, etc. And montan wax, especially refined montan wax, are also applied to precision casting, printing, daily chemical industry, and so on. But in the process of production of refined montan wax from montan wax, large amount of montan resin is generated as by-product and can not recycle so far [1-2]. Those by-products have not only caused the waste of resources, but also formed a kind of potential environmental pollutants. Previous investigations on chemical analysis of montan resin from Yunnan (China) by our group have showed many kinds of constituents, mainly including terpenoids, steroids, alkanes, and aromatic compounds by analysis methods of IR and GC-MS [3-7]. In order to further research of its way to ecological utilization, we studied their chemical constituents by modern chemical separation methods. In this study, fourteen compounds were isolated from the montan resin of Yunnan Esan (China), and their structures were elucidated by spectroscopic methods. They were identified as 31-homohopanoic acid (**1**), bacteriohopanetetrol (**2**), stigmaterol (**3**), 24,25-dinoroleana-1,3,5-triene-19,28-epoxy (**4**), bis-(7-methyl-nonane) phthalate (**5**), terephthalic acid-cyclic ethylene ester (**6**), 3,4-quinoxaline (**7**), 2-hydroxy-carboxyl-phthalic acidooctyl ester (**8**), dacane cyclohexane (**9**), methyl dodecyl (**10**), methyl heptyal (**11**), hendecane (**12**), decane (**13**), octane (**14**). Those fourteen compounds were isolated from montan resin for the first time.

EXPERIMENTAL SECTION

General methods

NMR spectra were recorded on Bruker AM 400 and DRX-500 NMR spectrometers (Bruker BioSpin Group, German) with tetramethylsilane (TMS) as an internal standard. EI-MS were measured on a VG Auto Spec-3000 mass spectrometer. Column chromatography (CC) was performed with silica gel (100-200 mesh, 200-300 mesh, Qingdao Marine Chemical Factory, Qingdao, China). Sephadex LH-20 (40-70 μ m, Amersham Pharmacia Biotech AB,

Uppsala, Sweden). HPLC was performed on an Agilent 1200 liquid chromatography using Zorbax SB-C18 (5 μ m, 9.4 mm \times 250 mm) semi-preparative column or Zorbax SB-C18 (4.6 mm \times 250 mm) analysis column with MeOH/H₂O in gradient. Preparative TLC (0.4-0.5 mm) was conducted on glass plates pre-coated silica gel GF₂₅₄ (Qingdao Marine Chemical Factory, Qingdao, China). Fractions were monitored by TLC plates, and spots were visualized by heating silica gel plates sprayed with 10 % H₂SO₄ in EtOH.

Experimental material

The montan resin from Esan Yunnan (China) were collected from TIANENGTONGAI Co., LTD.

Extraction and Isolation

The montan resin from Yunnan Esan (49.6 g) was extracted with petroleum ether and acetone to obtain 34.2 g petroleum ether extract and 13.3g acetone extract, respectively. The petroleum ether extract was subjected to silica gel column, eluted with petroleum-AcOEt (1:0-1:10) to provide Fr.I-IV. Fr.I (13.5 g) was further subjected to silica gel (100-200 mesh) and eluted with petroleum ether-AcOEt (35:1-1:10) to yield compound **11** (42.0 mg). Fr.II (7.4 g) was subjected to silica gel, eluted with petroleum ether-acetone (10:1-1:15), and then subjected to Sephadex LH-20, eluted with chloroform-MeOH (1:1) to yield compounds **2** (46.0 mg), **6** (20.1 mg), and followed by preparative TLC to give compound **4** (16.2 mg). Fr.III (4.9 g) was subjected to silica gel column and Sephadex LH-20, eluted with chloroform- MeOH (1:1) to yield compounds **7** (14.2 mg), **3** (20.3 mg). Fr.IV (5.0 g) was subjected to silica gel chromatography using eluent mixtures of petroleum ether- acetone, followed by preparative TLC to afford compounds **1** (38.4 mg), **14** (32.1 mg). The acetone extract was subjected to silica gel column, eluted with petroleum ether-acetone (1:0-1:10) to provide Fr.a-c. Fr.a (6.6 g) was subjected to repeated column chromatography on silica gel, eluted with petroleum ether-chloroform- MeOH and followed rechromatographed on Sephadex LH-20 with CHCl₃-MeOH (1:1) to yield compound **8** (14.2 mg). Fr.b (4.9 g) was subjected to silica gel column and Sephadex LH-20, eluted with chloroform-MeOH (1:1) to yield compounds **5** (20.3 mg), **13** (10.0 mg). Fr.c (6.5 g) was further subjected to silica gel (100-200 mesh), eluted with petroleum ether-AcOEt (35:1-1:10) to yield compounds **9** (26.4 mg), **10** (12.1 mg), **12** (25.0 mg).

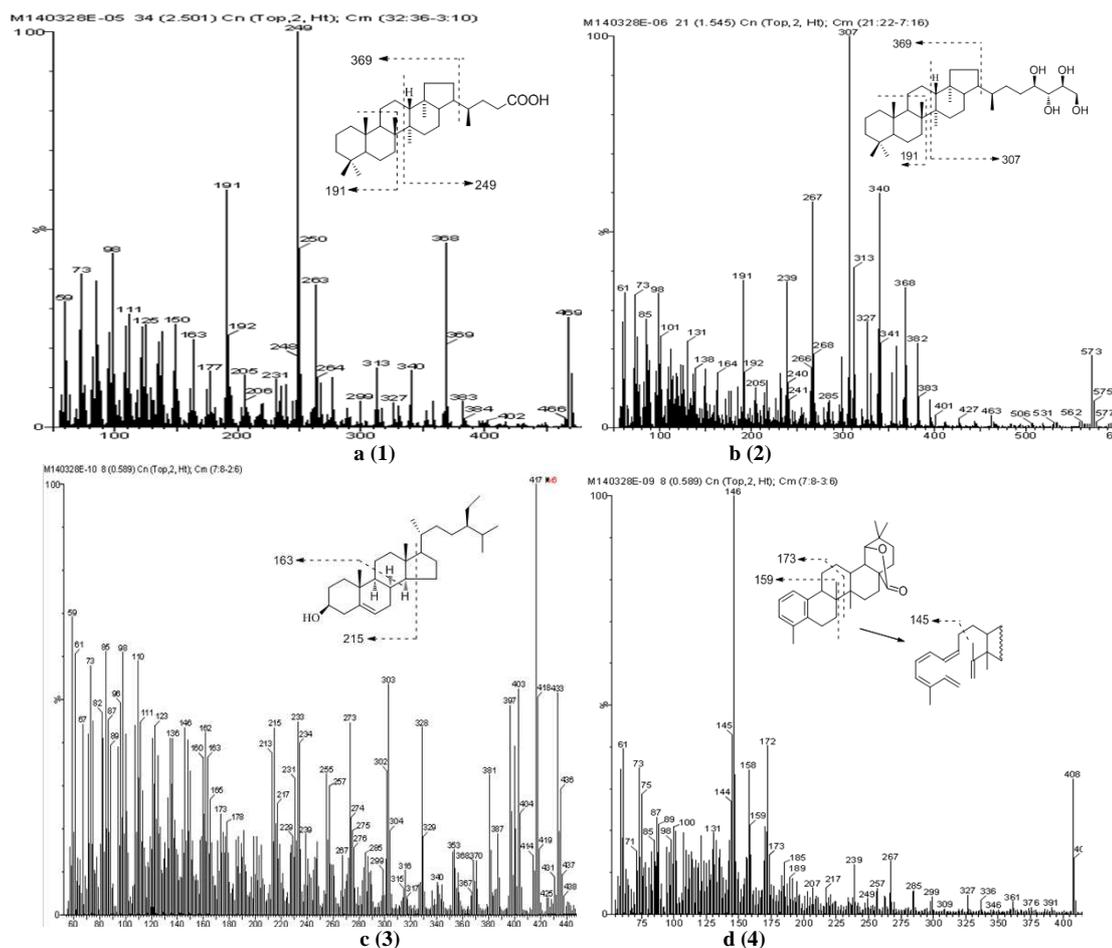


Fig.1. EI-MS data, corrected for background, of (a) 31-homohopanoic acid (1), (b) bacteriohopanetetrol (2), (c) stigmasterol(3), (d) 24,25-dinoroleana- 1,3,5-triene-19,28-epoxy (4)

EI-MS data of compounds (**1-4**) were shown in Fig. 1, and $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectroscopic data of compounds (**1-14**) were as below.

Compound 1 [8] white crystal, $\text{C}_{32}\text{H}_{54}\text{O}_2$, UV λ_{max} (MeOH): 210nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 0.65 (3H, s, H-25), 0.73 (3H, s, H-26), 0.76 (3H, s, H-28), 0.79 (3H, s, H-27), 0.89 (9H, s, H-23, 24, 29), 1.02-2.40 (32H, m, H1-H21), 3.58 (1H, d, $J = 6.2$ Hz, H-32), 3.65 (1H, dd, $J = 5.0, 12.5$ Hz, H-34), 3.69 (1H, t, H-35), 4.07 (1H, dd, $J = 7.0, 12.5$ Hz, H-33), 4.16 (1H, d, $J = 5.5$ Hz, H-35); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 40.2 (C-1), 20.8 (C-2), 42.0 (C-3), 33.1 (C-4), 56.0 (C-5), 18.6 (C-6), 33.2 (C-7), 41.5 (C-8), 50.3 (C-9), 37.3 (C-10), 22.6 (C-11), 23.8 (C-12), 49.1 (C-13), 41.7 (C-14), 33.5 (C-15), 27.3 (C-16), 54.3 (C-17), 44.2 (C-18), 41.5 (C-19), 30.7 (C-20), 45.7 (C-21), 36.2 (C-22), 33.3 (C-23), 21.4 (C-24), 15.8 (C-25), 16.3 (C-26), 16.4 (C-27), 15.6 (C-28), 31.0 (C-29), 19.5 (C-30), 33.1 (C-31), 177.2 (C-32).

Compound 2 [9] white crystal, $\text{C}_{35}\text{H}_{62}\text{O}_4$, UV λ_{max} (MeOH): 210 nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 0.65 (3H, s, H-25), 0.73 (3H, s, H-26), 0.76 (3H, s, H-28), 0.79 (3H, s, H-27), 0.89 (9H, s, H-23, 24, 29), 1.02-2.40 (32H, m, H1-H21), 3.58 (1H, d, $J = 6.2$ Hz, H-32), 3.65 (1H, dd, $J = 5.0, 12.5$ Hz, H-34), 3.69 (1H, t, H-35), 4.07 (1H, dd, $J = 7.0, 12.5$ Hz, H-33), 4.16 (1H, d, $J = 5.5$ Hz, H-35); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 40.2 (C-1), 20.8 (C-2), 42.0 (C-3), 33.1 (C-4), 56.0 (C-5), 18.6 (C-6), 33.3 (C-7), 41.6 (C-8), 50.3 (C-9), 37.3 (C-10), 22.7 (C-11), 23.9 (C-12), 49.2 (C-13), 41.7 (C-14), 33.6 (C-15), 27.5 (C-16), 54.4 (C-17), 44.3 (C-18), 41.5 (C-19), 29.9 (C-20), 45.9 (C-21), 36.7 (C-22), 33.3 (C-23), 21.5 (C-24), 15.8 (C-25), 16.4 (C-26), 16.5 (C-27), 15.7 (C-28), 31.6 (C-29), 19.9 (C-30), 33.2 (C-31), 70.5 (C-32), 82.9 (C-33), 75.4 (C-34), 72.42 (C-35).

Compound 3 [10] white crystal, $\text{C}_{29}\text{H}_{50}\text{O}$, UV λ_{max} (MeOH): 210nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 0.68 (3H, s, H-18), 0.80 (3H, s, H-21), 0.84 (3H, s, H-29), 0.82 (3H, d, $J = 7.5$ Hz, H-27), 0.92 (3H, d, $J = 8.0$ Hz, H-26), 1.0 (3H, s, H-19), 0.73-2.40 (30H, m, H1-H25), 2.02 (1H, s, H-25), 3.49 (1H, s, H-3), 3.57 (1H, m, H-3), 5.36 (1H, m, H-6); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 37.2 (C-1), 31.6 (C-2), 71.7 (C-3), 42.2 (C-4), 147.7 (C-5), 121.7 (C-6), 33.9 (C-7), 31.8 (C-8), 50.0 (C-9), 36.5 (C-10), 21.0 (C-11), 39.7 (C-12), 44.8 (C-13), 55.9 (C-14), 24.3 (C-15), 28.2 (C-16), 56.7 (C-17), 11.8 (C-18), 11.9 (C-19), 36.1 (C-20), 18.7 (C-21), 31.9 (C-22), 26.0 (C-23), 45.8 (C-24), 29.0 (C-25), 19.0 (C-26), 19.3 (C-27), 23.0 (C-28), 19.4 (C-29).

Compound 4 [11] white crystal, $\text{C}_{28}\text{H}_{38}\text{O}_2$, UV λ_{max} (MeOH): 210nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 0.71 (3H, s), 0.91 (3H, s), 1.00 (3H, s), 1.05 (3H, s), 2.20 (3 H, s, H-23), 7.12 (1H, d, $J = 8.0$ Hz, H-1), 7.06 (1H, t, H-2), 6.98 (1H, d, $J = 7.0$ Hz, H-3), 4.03 (1 H, s, H-19); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 123.2 (C-1), 125.4 (C-2), 127.0 (C-3), 140.1 (C-4), 135.9 (C-5), 24.6 (C-6), 25.9 (C-7), 33.6 (C-8), 40.59 (C-9), 134.6 (C-10), 25.8 (C-11), 25.0 (C-12), 46.6 (C-13), 38.56 (C-14), 28.82 (C-15), 31.9 (C-16), 46.3 (C-17), 36.1 (C-18), 85.9 (C-19), 37.9 (C-20), 32.4 (C-21), 28.6 (C-22), 19.6 (C-23), 13.0 (C-24), 13.0 (C-25), 28.8 (C-26), 24.0 (C-27), 179.9 (C-28).

Compound 5 [12] yellow powder, $\text{C}_{28}\text{H}_{46}\text{O}_4$, UV λ_{max} (MeOH): 365nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 7.71 (2H, dd, $J = 6.0, 9.5$ Hz, H-2, H-6), 7.53 (2H, dd, $J = 4.0, 8.5$ Hz, H-3, H-4), 4.30 (4H, t, H-2', 2''), 1.71 (4H, m, H-3', 3''), 1.56 (6H, m, H-8', 8'', H-9', 9''), 1.25 (16H, m, H3'-H7', H3''-H7''), 0.93 (6H, m, H-11', 11''), 0.88 (6H, m, H-10', 10'').

Compound 6 [13] yellow powder, $\text{C}_{16}\text{H}_{22}\text{O}_5$, UV λ_{max} (MeOH): 365nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 8.19 (1H, d, $J = 7.5$ Hz, H-6), 7.68 (1H, d, $J = 8.0$ Hz, H-3), 7.48 (1H, t, H-5), 3.49 (2H, t, H-3'), 1.56 (4H, m, H-3', H-4'), 1.25 (8H, m, H5'-H8'), 0.88 (3H, m, H-9').

Compound 7 [14] yellow powder, $\text{C}_{10}\text{H}_8\text{O}_4$, UV λ_{max} (MeOH): 365nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 4.69 (4H, s, H-3', H-4'), 8.10 (4H, s, H-2, H-3, H-6, H-5), $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 134.2 (C-1, 4), 128.0 (C-2, 3, 5, 6), 167.7 (C-1', 6'), 63.2 (C-3', 4').

Compound 8 [15] yellow powder, $\text{C}_{10}\text{H}_8\text{N}_2\text{O}$, UV λ_{max} (MeOH): 365nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 4.99 (4H, s, H-1, H-10), 7.55 (2 H, s, H-3, H-8), 8.71 (2H, s, H-5', 6'').

Compound 9 [16] white powder, $\text{C}_{16}\text{H}_{32}$, UV λ_{max} (MeOH): 254nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 0.86 (3H, m, H-7), 1.25 (10H, m, H2-H6), 1.61 (10H, m, H-cyclohexane).

Compound 10 [17] white powder, $\text{C}_{13}\text{H}_{26}\text{O}_2$, UV λ_{max} (MeOH): 254nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 0.87 (3H, m, H-7), 1.25 (4H, s, H3-H6), 1.56 (3H, m, H-2), 3.49 (3H, s, H-1).

Compound 11 [18] white powder, $\text{C}_8\text{H}_{16}\text{O}_2$, UV λ_{max} (MeOH): 254nm; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 0.87 (3H, m, H-7), 1.25 (4H, s, H3-H6), 1.56 (3H, m, H-2), 3.49 (3H, s, H-1).

Compound 12 [19] white powder, C₁₁H₂₄, UV λ_{max} (MeOH): 254nm; ¹H-NMR (CDCl₃, 500 MHz) δ: 1.25 (18H, m, H2-H10), 0.88 (6H, m, H-1, H-11).

Compound 13 [20] white powder, C₁₀H₂₂, UV λ_{max} (MeOH): 254nm; ¹H-NMR (CDCl₃, 500 MHz) δ: 1.25 (16H, m, H2-H9), 0.88 (6H, m, H-1, H-10).

Compound 14 [20] white powder, C₈H₁₈, UV λ_{max} (MeOH): 254nm; ¹H-NMR (CDCl₃, 500 MHz) δ: 1.27 (12H, s, H2-H7), 0.88 (6H, m, H-1, H-8).

RESULTS AND DISCUSSION

Aim to achieve utilization of the montan resin efficiently and environment-friendly, our group, on the basis of previous studies, conducts the in-depth research about the chemical compositions in montan resin produced in Yunnan Esan (China). Fourteen compounds were isolated from the montan resin by modern chemical separation methods, and the structures of fourteen compounds were elucidated by spectroscopic methods. They are 31-homohopanoic acid (1), bacteriohopanetetrol (2), stigmasterol (3), 24,25-dinoroleana-1,3,5-triene-19,28-epoxy(4), bis-(7-methyl-nonane) phthalate (5), terephthalic acid-cyclic ethylene ester (6), 3,4-quinoxaline (7), 2-hydroxy- carboxyl-phthalic acidooctyl ester (8), dacycane cyclohexane (9), methyl dodecyl (10), Methyl heptyal (11), hendecane (12), decane (13), octane (14). Our study not only further define the compositions of montan resin, but also offer the scientific basis for reasonable development and utilization of montan resin.

CONCLUSION

Those fourteen compounds (1-14) were isolated from montan resin for the first time. As we know, pentacyclic triterpenoids and steroids are accepted as active compounds in natural products. By separation, those compounds provided a reference for pharmaceutical development of montan resin.

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