MEDICINAL PLANTS AND HERBS OF NEWFOUNDLAND. PART 1. CHEMICAL CONSTITUENTS OF THE AERIAL PART OF PINEAPPLE WEED (*Matricaria matricarioides*)

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ABSTRACT

The aerial part of Pineapple weed (*Matricaria matricarioides*), an adulterant of Chamomile, was investigated for its chemical constituents. Nine compounds were isolated and identified as two spiroethers [*cis* - en - yn - dicycloether **1** and *trans* - en - yn - dicycloether **2**], three coumarins [7 - methoxycoumarin (Herniarin) **3**, umbelliferone **4** and 7 - methoxy - 3, 4 - dihydrocoumarin **5**], phytol **6**, luteolin - 7 - glucoside **7**, (*Z*) - 2 - β - D - Glucopyranosyloxyl - 4 - methoxycinnamic acid **8**, and (*E*) - 2 - β -D-Glucopyranosyloxyl - 4 - methoxycinnamic acid **9**. By GC-MS analysis, the major components of the steam distilled volatile oil were identified as *trans*-en-yn-dicycloether and *cis*-en- yn-dicycloether, with the *trans*-form being more abundant than the *cis*-form. The results indicated some similarities between the constituents of Pineapple weed and those of German Chamomile. All these nine compounds are reported for the first time from Pineapple weed growing in Newfoundland. Compound **5** is reported from this plant genus for the first time.

Keywords: Pineapple weed; Chamomile; *Matricaria matricarioides*; Chemical constituents; Spiroether.

INTRODUCTION

Chamomiles are important medicinal plants usually taken as aqueous infusion (chamomile tea) for minor digestive disorders and as mild sedatives. In addition to medicinal applications, extracts of the chamomiles are used as scent, nonirritating hair dye, etc. (1). There are two plant types of Chamomile, Roman Chamomile [*Chamaemelum nobile* (L.) All.], and German chamomile [*Matricaria recutita* L.], with the latter (often called matricaria) being more important commercially. Matricaria is mainly employed for its anti-inflammatory and spasmolytic properties. Also, extracts or the volatile oil of this plant are used in creams and ointments to treat inflammatory skin conditions, and as an antibacterial and antifungal agent (2). Some similar plants are used as adulterants of Chamomile. Pineapple weed [*Matricaria matricarioides* (less.)] is one of these adulterants (1). Because of the chamomiles' widespread use and economic significance, much chemical research has been conducted and many compounds have been isolated or detected from Chamomile (1). However, only few chemical research has been reported for Pineapple weed, such as the report of its polysaccharides (3), the isolation of umbelliferone (4) and the research on its volatile oil (5). As Pineapple weed is wildly spread in Newfoundland, Canada, and as it is an adulterant of Chamomile, we carried out this research to investigate its constituents and to compare them with the constituents of German chamomile.

MATERIALS AND METHODS

General: GC-MS was performed on an Agilent GC-MS system. NMR spectra were carried out on a Bruker AVANCE 500MHz spectrometer with TMS as internal standards. APCI mass spectra were measured on an Agilent 1100 series LC/MSD system. Silica gel used in column chromatography was provided by Sigma-Aldrich company. (Silica gel, Davisil, 100-200 mesh). Octadecyl-functionalized silica gel (ODS) was also obtained from Sigma-Aldrich company. Organic solvents for chromatography were obtained from Fisher Scientific.

Plant material: The plant material was collected in October, 2003, in the campus of Memorial University of Newfoundland, St. John's, NF, Canada. A voucher sample was deposited at the School of Pharmacy and was authenticated as the aerial part of *matricaria metricariodes* by Dr. Wilf Nicholls of the Botanical Garden of Memorial University of Newfoundland.

Volatile oil analysis: Steam distillation was performed on fresh samples of the plant (6.9g after dried). The steam was cooled, collected and extracted with CH_2Cl_2 (30 ml). One µl of the

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CH_2Cl_2 solution was applied to GC-MS for analysis of the volatile oil. GC conditions: column, DB-5, J@W Scientific, 0.25mm x 30 m; injection temperature, 280 °C, column temperature, 70 °C hold for 1 min then 20 °C/min to 200 °C hold for 1 min, then 15 °C/min to 280 °C hold for 1min; carrier gas, He; *trans* - en - yn - dicycloether R_t 10.89, Area percentage 27.3, MS *m/z*: 200 (M^+, 100%), 157 (30%), 115 (60%); *cis* - en - yn - dicycloether R_t 10.95, Area percentage 9.3, MS *m/z*: 200 (M^+, 100%), 157 (30%), 115 (60%); Other unidentified peaks R_t 8.21, Area percentage 14.3, MS *m/z*: 194 (20%), 179 (100%); R_t 8.93, Area percentage 16.0, MS *m/z*: 236 (20%), 221 (100%); R_t 15.60, Area percentage 24.1, MS *m/z*: 279 (M^+, 30%), 149 (100%) (possibly, the last peak is an oxidation product of the en-yn-dicycloethers produced at the high temperature of GC, as pure en-yn-dicycloethers also gave this peak).

Extraction and isolation: The aerial part of the Pineapple Weed (1.5 Kg) was extracted with MeOH under reflux. The solution was collected and concentrated under vacuum to give 100 g of the extract. It was suspended in water and extracted first with hexane, then chloroform, and finally ethyl acetate. Hexane, Chloroform and Ethyl Acetate extracts (18, 10 and 10 g, respectively) were chromatographed on Silica Gel and ODS column to get compounds **1** (1) (0.5 g), **2** (1) (1 g) and **6** (7) (3 mg) from the hexane soluble part, compounds **3**-**5** (6a) (20 mg, 3 mg and 5 mg, respectively) from the $CHCl_3$ soluble part, and compounds **7**-**9** (6b, 6c, 10) (10 mg, 8 mg and 2 mg, respectively) from the EtOAc soluble part.

Compound **1**, a brown oil, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.99 (3H, s, 6'-CH_3), 2.07 (2H, m, H-3a, 4a), 2.25 (1H, m, H-4b), 2.30 (1H, m, H-3b), 4.00 (1H, q, J=7.5Hz, H-2a), 4.24 (1H, dt, J=4.0, 8.5 Hz, H-2b), 4.60 (1H, s, H-1'), 6.14 (1H, d, J=6.0 Hz, H-9), 6.23 (1H, d, J= 6.0Hz, H-8); ^{13}C NMR δ 5.1 (C-6'), 24.8 (C-3), 36.0 (C-4), 65.5 (C-2'*), 70.0 (C-2), 71.1 (C-4'*), 77.1 (5'*), 79.2 (C-1'), 80.9 (C-3'*), 121.4 (C-5). 127.8 (C-8), 135.6 (C-9), 167.5 (C-7).

Compound **2**, a brown oil, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.00 (3H, s, 6'-CH_3), 2.12 (2H, m, H-3a, 4a), 2.18 (1H, m, H-4b), 2.25 (1H, m, H-3b), 4.00 (1H, q, J=7.5Hz, H-2a), 4.20 (1H, dt, J=4.0, 8.5 Hz, H-2b), 4.95 (1H, br, H-1'), 6.22 (1H, dd, J=1.5, 6.0Hz, H-9), 6.70 (1H, d, J= 6.0Hz, H-8); ^{13}C NMR δ 4.8 (C-6'), 24.7 (C-3), 36.0 (C-4), 65.1 (C-3'), 69.9 (C-2), 71.7 (C-4'*), 76.5 (C-2'), 79.8 (C-5'*), 80.0 (C-1') 121.0 (C-5). 126.1 (C-8), 136.0 (C-9), 169.0 (C-7). * Note: assignment may be exchanged. EI MS: 200 (M^+, 100%).

Compound **5**, a white powder, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.41(2H, t, J=7.5Hz, H-3), 2.66 (2H, t, J=7.5Hz, H-4), 3.65 (3H, s, OCH_3), 6.29 (1H, dd, J=3.0, 8.0 Hz, H-6), 6.36 (1H, d, J=3.0 Hz, H-8), 6.93 (1H, d, J= 8.0 Hz, H-5). ^{13}C NMR δ 24.9 (C-4), 34.1 (C-3), 54.8 (OCH_3), 101.2 (C-8), 103.9 (C-6), 119.3 (C-10), 130.0 (C-5), 155.5 (C-9), 158.6 (C-7), 174.2 (C-2). EI MS: 178(M^+, 100%).

Compound **8**, a colorless crystalline powder, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.13 (1H, m, H-4'), 3.26 (2H, m, H-2', 3'), 3.53 (overlapped with H_2O signal, H-5', 6'$_a$), 3.70 (1H, d, J=12.0 Hz, H-6'$_b$), 3.78 (3H, s, 4-OCH_3), 4.86 (1H, d, J=7.0Hz, H-1'), 5.77 (1H, d, J=12.5 Hz, H-8), 6.56 (1H, dd, J=2.5, 8.5 Hz, H-5), 6.73 (1H, d, J=2.5 Hz, H-3), 7.17 (1H, d, J= 12.5Hz, H-7), 7.70 (1H, d, J= 8.5Hz, H-6); ^{13}C NMR δ 50.3 (C-4-OCH_3), 60.7 (C-6'), 69.8 (C-4'), 73.3 (C-2'), 76.5(C-3'), 77.2 (C-5'), 100.8 (C-3, 1'), 117.0 (C-5), 116.7 (C-1), 118.3 (C-8). 131.4 (C-6), 136.4 (C-7), 156.7 (C-2), 161.2 (C-4), 167.7 (C-9). Negative API-MS: 355 (M^+-1, 100%).

Compound **9**, a colorless crystalline powder, ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.14 (1H, m, H-4'), 3.53 (overlapped with H_2O signal, H-2', 3', 5', 6'$_a$), 3.69 (1H, d, J=12.0 Hz, H-6'$_b$), 3.76 (3H, s, 4-OCH_3), 4.99 (1H, d, J=6.0Hz, H-1'), 6.39 (1H, d, J=16.0 Hz, H-8), 6.59 (1H, d, br., J=8.5 Hz, H-5), 6.74 (1H, s, br., H-3), 7.61 (1H, d, J= 8.5 Hz, H-6), 7.80 (1H, d, J= 16.0 Hz, H-7); ^{13}C NMR δ 55.0 (C-4-OCH_3), 60.0 (C-6'), 69.0 (C-4'), 73.0 (C-2'), 76.0(C-3'), 77.0 (C-5'), 100.5 (C-3, 1'), 101.0 (C-3), 108.0 (C-5), 117.0 (C-1), 118.0 (C-8), 130.0 (C-6), 139.0 (C-7), 157.0 (C-2), 161.0(C-4), 170.0 (C-9).

RESULTS AND DISCUSSION

A methanol extract of Pineapple weed was subjected to repeated column chromatography to get nine compounds. The structures of these compounds were determined by comparing their spectral data with those reported or analyzing their various 2D-NMR spectral data. Among these compounds, two were identified as Spiroethers [*cis*-en-yn-dicycloether **1**, and *trans*-en-yn-dicycloether **2**], three were identified as coumarins [7-methoxycoumarin (Herniarin) **3**, umbelliferone **4** and 7-methoxy-3,4-dihydrocoumarin **5**], two were identified as cinnamic acid derivatives [(*Z*)-2-*β*-D-Glucopyranosyloxyl-4-methoxycinnamic acid **8**, and (*E*)-2-*β*-D- Glucopyranosyloxyl- 4 - methoxycinnamic acid **9**], one was identified as flavonoid [luteolin-7- glucoside **7**], another one was a diterpene (phytol) **6**.

Compound **1**, a brown oil, exhibited an $[M]^+$ ion peak at *m/z* 200 in EI-MS. In the ^{1}H NMR spectrum of **1**, totally 12 proton signals including a singlet methyl signals were observed.

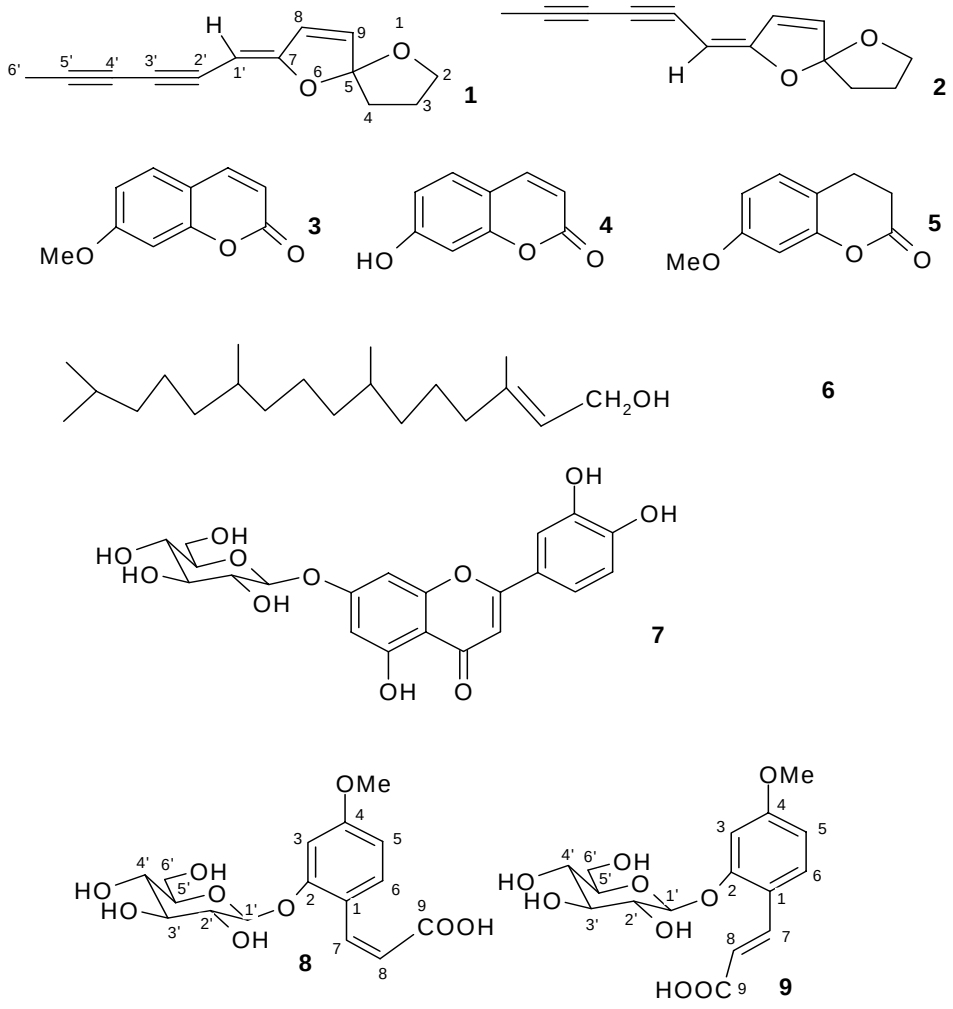

Fig 1. Structures of compounds isolated from pineapple weed.

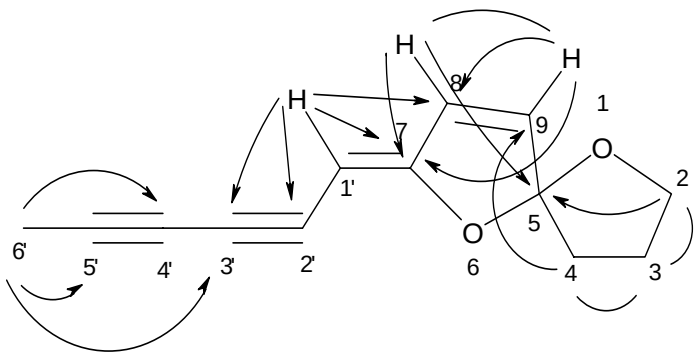

Fig. 2. Important HMBC ( ) and COSY ( ) correlations in the spectra of compound **1**

In the ^{13}C NMR, compound **1** displayed 13 carbon signals. The much less proton numbers as regard to the carbon numbers suggests that this compound is highly unsaturated or carries multi-epoxy moieties. Considering its molecular weight of 200, it is deduced that this is not a multi-epoxy carrying molecule but is highly unsaturated and its molecular formula was deduced as $C_{13}H_{12}O_2$. In the ^{13}C NMR spectrum, only four carbon signals were more down-fielded than δ 100, while six carbon signals appeared between δ 60 and 80, suggesting the existence of acetylenic group(s) in the molecule. With the aid of COSY and HMQC spectra, all the proton signals were assigned to their corresponding carbons. Further analysis of the COSY and HMBC spectra of compound **1**, made it possible to determine the planar structure as shown in figure 2.

In a NOESY experiment, significant NOE effect was observed between H-1' and H-8 signals, therefore, the stereochemistry at C-1' and C-7 of compound **1** is determined as *cis* form. Based on the above evidences, compound **1** was identified as *cis*-en-yn-dicycloether.

Similarly, compound **2** was identified as *trans*-en-yn-dicycloether, where no NOE effect was observed between H-1' and H-8 signals.

Coumarins **3** and **4** were identified as Herniarin and, umbelliferone, respectively, by comparing their ^{13}C NMR spectra with those reported in the literature (6a).

Compound **5** was identified in nearly pure form, but contaminated with umbelliferone. It gave a molecular weight of 178 in EIMS. Its ^{1}H NMR, after deduction of the umbelliferone signals, displayed two sets of triplet signals at δ 2.41(2H, t, J=7.5Hz) and 2.66 (2H, t, J=7.5Hz), attributed to H-3 and H-4. A singlet signal integrated for 3 protons appeared at δ 3.65 (3H, s, OCH_3). A pattern of 1, 2, 4-tri-substituted benzene signals appeared at δ 6.29 (1H, dd, J=3.0, 8.0 Hz), 6.36 (1H, d, J=3.0 Hz), 6.93 (1H, d, J= 8.0 Hz). These data suggested that compound **5** is 7-methoxy-3, 4-dihydrocoumarin. Its structure was finally confirmed by 2D NMR spectra.

Compound **6** was identified by comparing its NMR data with that reported for phytol (7). Phytol was detected in German Chamomile by GC-MS. in recent years (8, 9).

Compound **7** was identified as luteolin-7-glucoside by analyzing its 2D NMR and comparing its ^{13}C NMR spectral data with those reported in the literature (6b, 6c).

Compound **8**, was a colorless crystalline powder. In its ^{1}H NMR, a group of proton signals characteristic for 1, 2, 4-trisubstituted benzene ring were appeared at δ 6.56 (1H, dd, J=2.5, 8.5 Hz, H-5), 6.73 (1H, d, J=2.5 Hz, H-3), 7.70 (1H, d, J= 8.5Hz, H-6); A pair of doublet signals with relatively large coupling constant appeared at δ 5.77 (1H, d, J=12.5 Hz, H-8) and 7.17 (1H, d, J= 12.5Hz, H-7); and some sugar signals at δ 3-5. The sugar part was identified as glucose by comparing its ^{13}C NMR data with those of β-methyl-D-glucopyranoside (6c). The anomaric cofiguration was determined as β-form according to its large coupling constant δ 4.86 (1H, d, J=8.0Hz, H-1'). By further analysis of its 2D-NMR spectra, the position of the substitutes were determined as 2-β-D-Glucopyranosyloxyl-4-methoxycinnamic acid. The configuration of the C-7/C-8 double bond was determined as *cis* by the coupling constant of H-7 and H-8. Based on the above evidence, the structure of compound **8** was determined as (*Z*)-2-β-D-Glucopyranosyloxyl-4-methoxycinnamic acid Similarly, compound **9** was identified as (*E*)-2-β-D-Glucopyranosyloxyl-4-methoxy-cinnamic acid, where a larger coupling constant of H-7 and H-8 was observed (J=16.0 Hz). GC-MS analysis of a steam distilled volatile oil revealed that its major components are *cis*-en-yn-dicycloether and *trans*-en-yn-dicycloether, with the *trans*-form being more abundant than the *cis*-form. It should be noted that in a research on the volatile oil of *M. matricarioides*, the spiroethers were not reported among the major constituents (5), suggesting that the constituents of volatile oil might change as the collecting time or growing place of the plants change. The major constituents of German Chamomile oil were reported to be α-bisabololoxide B and α-bisabololoxide A (1), which were not detected among the major components in the volatile oil of pineapple weed in our experiment.

Jain *et al* has reported the isolation of 7-methoxycoumarin (**3**) from Pineapple weed collected on the Cordon Head Campus of University of Victoria (4). In our present investigation on the Pineapple Weed collected in Newfoundland, eight other compounds (**1**, **2**, **4**-**9**) were also isolated together with 7-methoxycoumarin. This is the first report of 7-methoxy-3, 4 - dihydrocoumarin **5** from this plant genus.

All isolated compounds, except for compound **5**, have been reported from German Chamomile(1, 8-10). The spiroethers have been reported to have antispasmodic and antiinflammatory activities with the *cis* form being more pharmacologically potent than the *trans* form (1). Compounds **8** and **9** were reported as the main components of water–alcoholic extracts of German Chamomile (10).

The above results showed that the chemical constituents of Pineapple Weed growing in Newfoundland are very similar to those of German Chamomile. On the other hand, some differences in the type and quantities of their constituents also exist. Consequently, it is suggested from the chemical point of view, that Pineapple Weed grown in Newfoundland may be used as a medicinal plant similar to Chamomile but it may also has its own indications.

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