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► **To cite this version:**

Gersende Berthier, Sen Dou, Torren Peakman, Eric Lichtfouse. Identification of bound alcohols in soil humic acids by gas chromatography-mass spectrometry. *European Journal of Mass Spectrometry*, 2000, 6 (5), pp.439-441. 10.1255/ejms.368 . hal-00263408

**HAL Id: hal-00263408**

**<https://hal.science/hal-00263408>**

Submitted on 12 Mar 2008

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Revised version

European Journal of Mass Spectrometry 6, 439-441, 2000. doi: 10.1255/ejms.368

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## Identification of bound alcohols in soil humic acids by GC-MS

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### Abstract

Humic acids are complex, partly macromolecular, yellow-brownish substances occurring in soils, waters and sediments. In order to bring some light on their molecular structure, crop humic acids were cleaved by alkaline hydrolysis (KOH). The products were fractionated by thin layer chromatography to give mono-alcohols which were analysed as acetate derivatives by gas chromatography coupled to mass spectrometry. Linear alcohols, sterols, stanols and plant-derived triterpenoid alcohols were identified by co-injection of pure standards and by comparison with literature data. These findings imply that alcohols could have been incorporated into the humic matrix by esterification with carboxylic acids. Furthermore, the presence of stanols as hydrogenated counterparts of sterols suggests that a process of hydrogenation is operating in soils.

### INTRODUCTION

Humic substances represent a major pool of organic carbon occurring in soil, sediment and waters<sup>1-2</sup>. It is generally accepted that humic acids are characterised as polydispersed, acidic, amorphous substances ranging in molecular mass from a few hundred to several thousand daltons. However, the pathways of formation of humic acids remains an enigma mainly because of the lack of structural identification of its network at the molecular level. To unravel the molecular structure of complex, high molecular weight natural organic substances such as kerogen, asphaltene, coal and humic substances, various chemical and physical methods have been applied<sup>3-5</sup>. In particular, the breakdown of humic substances by chemical and enzymatic degradation can release substantial amounts of compounds amenable by gas chromatography coupled to mass spectrometry<sup>5-7</sup>. In a recent investigations, we identified alkane and alkene biomarkers released by pyrolysis of humin<sup>8-12</sup>. Here, we report the identification of *n*-alkanols, stanols, sterols and plant-derived triterpenoid alcohols released by mild alkaline degradation of soil humic acids from crop soils.

### EXPERIMENTAL

Labware decontamination procedures are described elsewhere<sup>13</sup>. Soils cultivated for 23 years with maize (*Zea mays*) or wheat (*Triticum aestivum*) were sampled at Boigneville, France, then dried and sieved to 2 mm. 2 g of humic acids were extracted with 0.1 M sodium hydroxide under nitrogen to prevent oxidation of organic matter, precipitated at pH 1.5 with HCl, dialysed against water to remove molecules smaller than 2000 daltons, freeze-dried, extracted with diethyl ether to remove free organic compounds then refluxed 16 h. in 5 M potassium hydroxide-methanol under nitrogen. After addition of water, alcohols were extracted with diethyl ether (3 x), Na<sub>2</sub>SO<sub>4</sub>-dried then fractionated by thin layer chromatography (TLC) on silica-gel eluting methylene chloride with cholesterol as reference ( $R_F \sim 0.13$ ) to yield a mono-alcohol fraction which was then derivatised with an excess of acetic anhydride and pyridine (50°C, 1 h). The

products were fractionated by TLC on silica-gel eluting methylene chloride with acetates of cholesterol, ergosterol and *n*-hexadecanol as references ( $R_F \sim 0.70$ ) to yield a mono-acetate fraction which was then analysed by GC-MS. GC conditions: HP 5890 series II, on-column injector, 50 m x 0.32 mm i.d. capillary column coated with 0.52 mm 100% polymethyl-siloxane phase (Ultra 1), Helium carrier gas: 0.66 b. head pressure at 50°C (constant flow mode), oven temperature: 50-100°C (15°/min.), 100-310°C (3°/min.), 310°C (60 min.). MS conditions: HP 5989A quadrupole, electronic impact (70 eV), scanning from 50 to 700 amu.

## RESULTS AND DISCUSSION

The GC-MS total ion current trace of mono-alcohols, as acetate derivatives, released by alkaline hydrolysis of humic acids from a maize crop soil is presented on **Figure 1**. Three classes of alcohols were identified in hydrolysis products of humic acids by mass spectrometry and by comparison with pure standards: linear alkanols, sterols, stanols and triterpenoid alcohols (**Figure 2**). The spectra of all compounds show low relative intensity of the molecular ion and high relative intensity of the M-60 peak corresponding to the easy removal of acetic acid. Sterols give a high  $m/z$  255 peak due to the cleavage of the side-chain. The  $m/z$  215 base peak of stanols is explained by the cleavage of the 5-carbon ring.  $\beta$ - and  $\alpha$ -amyrin are easily characterised by a  $m/z$  218 base peak. Compounds **1,2,4,6-8,10** and **11** were identified by co-injection of pure standards. The structure of the other compounds were assigned on MS grounds and by comparison with literature data<sup>14-19</sup>.

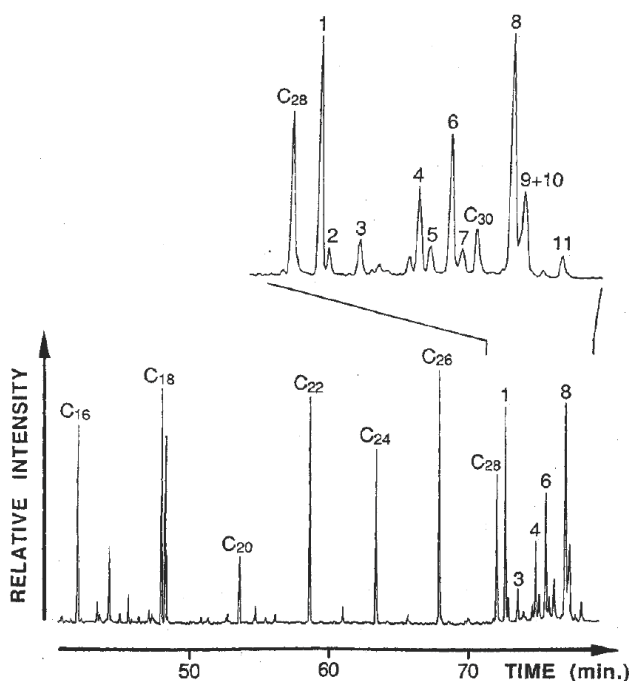


Figure 1. GC-MS total ion current of alcohols (as acetate derivatives) released by alkaline hydrolysis of humic acids from a maize crop field. A similar fingerprint was obtained by analysis of humic acids from wheat crop soils. C<sub>16</sub>-C<sub>30</sub> refer to *n*-alkanols. **1** Cholest-5-en-3 $\beta$ -ol (cholesterol), **2** 5 $\alpha$ -cholestan-3 $\beta$ -ol, **3** (3 $\beta$ , 22E)-ergosta-5,22-dien-3-ol (brassicasterol), **4** (24R)-ergost-5-en-3 $\beta$ -ol (campesterol), **5** (5 $\alpha$ , 24R)-ergostan-3 $\beta$ -ol, **6** (3 $\beta$ , 22E)-stigmasta-5,22-dien-3-ol (stigmasterol), **7** (3 $\beta$ ,5 $\alpha$ ,22E)-stigmast-22-en-3-ol, **8** stigmast-5-en-3 $\beta$ -ol ( $\beta$ -sitosterol), **9** olean-12-en-3 $\beta$ -ol ( $\beta$ -amyrin), **10** 5 $\alpha$ -stigmastan-3 $\beta$ -ol **11** urs-12-en-3 $\beta$ -ol ( $\alpha$ -amyrin).

The identification of bound alcohols in soil humic acids has several implications. First, a higher plant source<sup>20</sup> can be inferred for most alcohols, e.g. odd-carbon number *n*-alkanols, campesterol, stigmasterol,  $\beta$ -sitosterol,  $\beta$ -amyrin and  $\alpha$ -amyrin. Whereas cholesterol is probably derived at least partly from other organisms, as suggested by the higher  $\beta$ -sitosterol/cholesterol ratio of 8 in maize than in maize soils (value: 2)<sup>21</sup>. Second, the most likely pathway of incorporation of plant alcohols into soil humic acids could involve esterification of alcohols with carboxylic groups. This hypothesis is in agreement with the high concentration of acidic groups in humic substances<sup>1,5</sup>. Third, we identified C<sub>27</sub>-C<sub>29</sub> stanols (**2,5,9**) as hydrogenated counterparts of C<sub>27</sub>-C<sub>29</sub> sterols (**1,4,8**) in hydrolysis products of humic acids. Since stanols are rare in living organisms, their occurrence in soil suggests their derivation from sterols by hydrogenation into the soil. This assumption is supported by an investigation showing the hydrogenation of <sup>14</sup>C-cholesterol in sediments<sup>22</sup>.

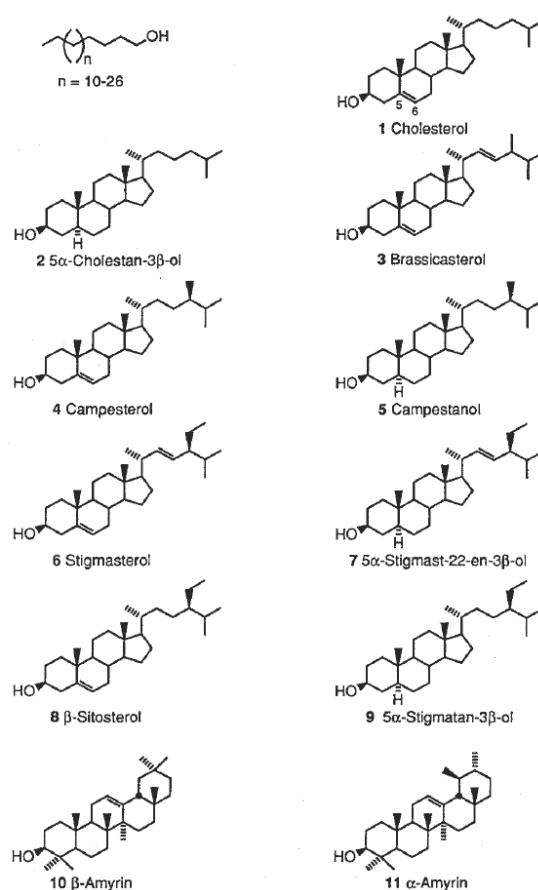


Figure 2. Molecular structure of alcohols released by alkaline hydrolysis of humic acids.

## CONCLUSION

Several classes of alcohols have been identified by GC-MS as bound moieties of soil humic acids using alkaline degradation. They are probably incorporated in the humic matrix by esterification with carboxylic groups. Most alcohols are typical of plant substances whereas cholesterol and cholestanol are probably inherited at least partly from other organisms. Furthermore, the presence of stanols in humic substances could be explained by hydrogenation of their sterols counterparts.

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