TRYPANOCIDAL ACTIVE COMPOUNDS FROM SCOTTISH ABIES NOBILIS AND PINUS SYLVESTRIS

Jamal Elmezogi*, Carol Clements, Veronique Seidel and Alexander Gray
Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK

Article Received on: 16/12/12 Revised on: 01/01/13 Approved for publication: 01/02/13

*Email: mezogi@yahoo.co.uk

ABSTRACT

Three active compounds obtained from the aerial parts of Abies nobilis and Pinus sylvestris isolated by different chromatographic techniques. Their structures were identified by NMR (H, 13C, COSY, HMBC) spectroscopic and mass spectrometric data and established as catechin, dehydroabietic acid and Dihydroconiferyl alcohol. The isolated compounds were exhibited activity against blood stream form of parasite Trypanosoma brucei brucei (S 427).

KEY WORDS: Pinaceae, Abies nobilis, Pinus sylvestris, Flavanol, diterpene, cinnamic acid, NMR, Antitryptapical activity

INTRODUCTION

The family Pinaceae is the second largest family in geographical range after the Cupressaceae. It contains 220 species and 11 genera, found mainly in the northern but also in southern hemisphere. The Pinaceae family originates in the southern hemisphere. The Pinaceae family originates in the order Pinales, and consists of 4 subfamilies: the Pinoideae, Pinioideae, Laricioideae and Abietoideae.

Humans have at all times used plants in a multitude of applications in a tradition spanning human development. Trypanosomiasis is an major public health problem in various African populations who are exposed to sleeping sickness and tourists are also at risk over 66 million people in 36 countries of sub-Saharan Africa are under the threat of Human African Trypanosomiasis (HAT) and each year an average of 500,000 people are infected with HAT and 50,000 deaths are reported. The reported resistances to the available drugs for different species of the parasite and relapse for unknown reasons suggest that there is an urgent need to search for alternative sources of drugs.

Chromatographic techniques were applied for the isolation of catechin (1), dehydroabietic acid (2) and dihydroconiferyl alcohol (3) from the aerial parts of Abies nobilis and Pinus sylvestris. In this paper, we report the isolation, identification and trypanocidal activities of three compounds from the aerial parts of Abies nobilis and Pinus sylvestris.

MATERIAL AND METHODS

Plant Aerial parts of Abies nobilis were purchased from Albartrees, UK in October 2003 and the aerial parts of Pinus sylvestris were collected from Whim bog in Edinburgh, Scotland in April 2006 materials. Herbarium specimens were deposited at the Phytochemistry Laboratory at the University of Strathclyde where they were identified and given the following voucher numbers ABNO1003 (Abies nobilis), PSY1003 (Pinus sylvestris).

EXTRACTION AND ISOLATION OF PURE COMPOUNDS

The aerial parts materials were extracted in a Soxhtlet apparatus by using different solvent systems starting from non-polar n-hexane (60-80°C), semi polar chloroform or ethyl acetate and finally polar solvent as methanol for 2 to 3 days. The extracts were concentrated using a rotary evaporator (BUCHI Labortecnik AG, Switzerland) under reduced pressure at a maximum temperature of 50°C and stored at -20°C before use. A range of chromatographic methods were used; such as Column Chromatography (CC) using silica gel (Kieselgel 60; 0.063-0.200mm, Merck), Sephadex LH-20 (Sigma-Aldrich, UK), Vacuum Liquid Chromatography (VLC) using silica gel (Kieselgel 60H PF, VWR International Ltd, UK) and Flash Chromatography [Flash Master Personal] TM. NMR spectroscopy was extensively used to elucidate the structures of isolated compounds, IR spectra of samples were recorded in an automatic IR spectrophotometer in the solid state as pressed potassium bromide (KBr) discs, HRESIMS of all isolated compounds was performed in FTMS-Orbitrap (ThermoFinnigan Bremen, Germany) to give the exact mass of the molecular ion that is useful to determine the molecular formula, Specific rotation of compounds with optical activity was measured by an automatic polarimeter Auto pol V. Trypanosoma brucei (S 427).

Test organism Trypanosoma brucei brucei (S 427) was obtained from Prof. Mike Barratt, Glasgow University.

Preparation of sample and plate

The samples were dissolved in DMSO in such a way that the concentration was 10 mg/ml. 4μl of test solutions were added into column 2 of the plate. A 196μl of HMI-9 medium was introduced in all wells of the microplate. Serial dilution was carried out from well 2 to 11 which followed half fold dilution in the consecutive well from left to right. A solution of suramin (Sigma) was prepared in filtered sterilized water and had a concentration of 100μM. A 2μl of this solution was added in the wells of 12th column, 100μl suspension of blood stream form of T. b. brucei S427 (2-3X10⁴ trypanosomes/ml) was added to each well. The plate was then placed in an incubator at 37°C under 5% CO₂ humidified air. After a 48h incubation period, 20μl of the REDOX indicator Alamar blue was added to each well and then incubated again for a period of 24hrs. Fluorescence was determined in Wallac Victor at excitation and emission wavelengths of 530 and 590nm, respectively. The protocol was modified according to the previous paper.

RESULTS AND DISCUSSION

The biological activities of the isolated compounds were assessed against T.b.brucet and the test results were assessed...
The analysis of the ethyl acetate part of the methanolic extract, n-hexane extract of Abies nobilis and the chloroform extract of Pinus sylvestris using column and flash chromatography separations, led to the isolation of three active natural products such as catechin, dehydroabiatic acid and dihydroconiferyl alcohol. The structures were elucidated by mainly NMR spectroscopic and MS spectrometry. The activities of isolated compounds were assessed by Alamar Blue Assay.

Isolated compounds; catechin 1, dehydroabiatic acid 2, dihydroconiferyl alcohol 3 and standard drug (suramin) as a positive control, MIC (Minimum Inhibitory Concentration). Compound 1 was isolated from the ethyl acetate part of the methanolic extract of Abies nobilis. The HRESI-MS showed an [M+H]+ at m/z 291.0220 for the molecular formula of the compound as C15H14O6. The 1H and 13C NMR data [400MHz, CD3OD, Table 1] of compound 1 revealed to be catechin and confirmed this indication by the meta-coupled aromatic signals at δ 5.84 and δ 5.91 for ring-A, the ABX spin aromatic system (δ 6.75, 6.70 and 6.82) for ring-B and the characteristic signals at δ 2.52, 2.84, 3.96 and 4.55 for the flavanol ring C. In the 13C NMR spectrum, aromatic ring system, two oxygen-bearing methines and one methylene were observed among 15 signals, suggesting the flavanol-type compound. The sample yield was low and the optical rotation was not recorded. Hence, the absolute stereochemistry could not be confirmed. However, the large coupling constant between H-2 and H-3 indicated a trans diaxially relative stereochemistry. Therefore, The structure of compound 1 was identified as catechin (1) as compared with the literature data (Benavides et al., 2006).

<table>
<thead>
<tr>
<th>Position</th>
<th>1H (δ)</th>
<th>13C (δ)</th>
<th>1H (δ)</th>
<th>13C (δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.55 (d, J= 8.0Hz)</td>
<td>81.7</td>
<td>5.91 (d, J= 2.2Hz)</td>
<td>96.1</td>
</tr>
<tr>
<td>2</td>
<td>3.96 (d, J= 5.2, 8.0Hz)</td>
<td>67.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.52 (dd, J=16.2, 8.0Hz)</td>
<td>27.4</td>
<td>10</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>2.84 (dd, J= 16.2,5.2Hz)</td>
<td></td>
<td>1'</td>
<td>131.0</td>
</tr>
<tr>
<td>4</td>
<td>157.3</td>
<td>3'</td>
<td>2'</td>
<td>115.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.82 (d, J= 1.7Hz)</td>
</tr>
<tr>
<td>5</td>
<td>5.84 (d, J= 2.2Hz)</td>
<td>97.0</td>
<td>5'</td>
<td>6.75 (d, J= 8.5Hz)</td>
</tr>
<tr>
<td>6</td>
<td>157.0</td>
<td>6'</td>
<td></td>
<td>6.70 (dd, J=1.7Hz, 8.5Hz)</td>
</tr>
</tbody>
</table>

Figure 1: MICs in μM of isolated compounds against T. b. brucei
Compound 2 was isolated from VLC fraction of n-hexane extract of Abies nobilis. The HRESI-MS indicated a molecular ion at m/z 300.2087 which was analyzed for the molecular formula C_{20}H_{30}O_{2} IR (KBr) absorption at 1464, 2649, 2927 cm\(^{-1}\) were indicative of the presence of C=C, O-H and C-H groups stretching respectively.

The \(^1\)H NMR spectrum [400 MHz, CDCl\(_3\), Table 2] showed aromatic, aliphatic protons and 4 methyls; two appearing as equivalent doublets at \(\delta 1.21\) ppm (each, \(J=7.0\) Hz, Me-16, Me-17) and two other methyl groups attached to quaternary carbons and appearing as singlets at \(\delta 1.27\) ppm (Me-18) and \(\delta 1.20\) ppm (Me-20). In the aromatic region of the spectrum it showed a doublet at \(\delta 7.16\) \((J=8.0\) Hz, H-11), doublet at \(\delta 6.88\) \((J=1.2\) Hz, H-14) and doublet of doublets at \(\delta 6.99\) \(J=1.8\) Hz, 8.0Hz, H-12) indicating an ABX substitution pattern. A septet proton at \(\delta 2.88\) ppm (H-15) showed COSY correlation to two methyl groups (Me-16 and Me-17) whereas these methyls also showed COSY correlation to each other indicating the presence of an isopropyl group. In \(^{13}\)C NMR data [100MHz, CDCl\(_3\), Table 2] showed 20 carbon atoms including one at \(\delta 184.5\) ppm due to a carbonyl function. This number of carbon atoms corresponds to diterpene moiety which contains a carboxylic acid group and 4 methyls. Two methyls at positions 16 and 17 showed HMBC correlations to the aromatic ring and another methyl (\(\delta 1.27\)ppm) gave a correlation to the carboxylic acid. Some methylenes (\(\delta 1.55, 1.87, 2.87 \) and 2.93) exhibited HMBC correlation to the aromatic ring whereas some other methylenes (\(\delta 1.68 \) and 1.83) showed correlation to the carboxylic acid and one methyl (\(\delta 1.27\)). The optical rotation of compound 2 was found to be \([\alpha]_D = +57\) which is comparable with the value found in the literature (Kayoko et al., 2008). Further comparisons of the NMR data to those of the literature identified that the compound is dehydroabietic acid (2). This is the first report of this compound being found from this plant. The experimental data were in agreement with the published data (Gigante et al., 1995).

Compound 3 By using VLC and further purification over sephadex LH20 column chromatography and the flash chromatography, JE7 was isolated from the chloroform extract of Pinus sylvestris. Compound 3 solved for C_{10}H_{16}O_{3} according to HRESI-MS at m/z 182.0943. \(^1\)H NMR spectrum [400 MHz, CDCl\(_3\), Table 3] showed signals for both aromatic and aliphatic protons. The aliphatic protons (H-7, H-8 and H-9) resonated from \(\delta 1.86\) to 3.62 ppm and methoxy group at \(\delta 3.82\) as a singlet peak was observed. From the HMBC NMR data of compound 3 in CDCl\(_3\) (Table 3): 10 signals were observed and a methoxy group at 55.9ppm was present. By using \(^1\)H NMR and \(^{13}\)C NMR spectroscopy and comparing with the previous data (Ma et al., 2000), compound 3 was identified as dihydroconiferyl alcohol (3).

### ACKNOWLEDGEMENTS

This investigation was carried out in Phytochemistry laboratory at University of Strathclyde and supported by Secretary of Education, Libya. We thank Dr. David Adam and Dr Jamie Tweedy, Department of Chemistry, University of Glasgow for NMR and MS experiments and Mrs. Carol Clements for process the bio-activity studies in SIDR laboratories at University of Strathclyde, Glasgow, UK.
REFERENCES

Source of support: Nil, Conflict of interest: None Declared