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GALLOTANNINS FROM QUERCUS ROBUR CULTIVATED IN EGYPT

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في هذا البحث تم فصل أربعة مركبات جالوتانينية بالإضافة إلى حامض الجاليك وميثوكسى حامض الجاليك من خلاصة خلات الإيثيل لبذور الكويركس روبر. أثنان من هذه المركبات تفصل لأول مرة من العائلة الفاجيسية التابع لها النبات.

تم فصل هذه المركبات بإستخدام كروماتوجرافيا العمود المعبأ بمادة ODS ثم MCI-gel CHP-20P وإستخدام نسب مختلفة من الميثانول والماء كمذيب. وقد تم التعرف على هذه المركبات بإستخدام التقنيات الحديثة من الرنين النووى المغناطيسى البروتونى والكربونى ذات الإتجاه الواحد والاتجاهين وكذلك مطياف الكتلة.

Four gallotannins: 1,2,3-tri-O-galloyl-β-D-glucose; 1,2,3,6-tetra-O-galloyl-β-D-glucose; 1,2,3,4,6-penta-O-galloyl-β-D-glucose and 1,2,3,4,6-penta-O-galloyl-α-D-glucose, together with gallic acid and methoxy gallic acid have been isolated from the seeds of Quercus robur and their structures were elucidated on the basis of chemical and spectral evidence.

INTRODUCTION

Quercus robur L. is a majestic tree which can reach a height of 50 m. The leaves are alternate, simple, deciduous, herbaceous, ovate - oblong with 5-7 pairs of broad lobes and their color is dark green on the upper side and paler below.[1,2] The fruits are green to brown acorns.

A decoction of of the bark of *Q. robur* is used as a gargle to treat sore throats and tonsilitis. It is also used as a wash, lotion, or ointment to treat hemorrhoid, anal fissures, small burns, and other skin problems. Less commonly, a decoction of the bark is taken in small doses to treat diarrhea, dysentry, and rectal bleeding. Powdered oak bark may be sniffed to treat nasal polyps, or sprinkled on eczema to dry the affected area. Oak galls are very astringent. They are used in small quantities, in place of bark.[1]

Quercus spp. (Fagaceae), have been studied and shown to produce a wide variety of hydrolysable tannins,[3-9] procyanidin glycosides and proanthocyanidin dimers and polymers,[10] catechin dimers, oligomers linked together via A- to B- ring biphenyl bonds.[11-14]

The heartwood of *Q. robur* is known to contain 10% by wt of ellagitannins.[15,16] In this study, we report for the first time four gallotannins in the seeds of this plant: 1,2,3-tri-O-galloyl-ß-D-glucose; 1,2,3,6-tetra-O-galloyl-ß-D-glucose; 1,2,3,4,6-penta-O-galloyl-ß-D-glucose and 1,2,3,4,6-penta-O-galloyl-α-D-glucose, together with gallic acid and methoxy gallic acid, two of them being reported for first time in family Fagaceae.

EXPERIMENTAL

General methods

Optical rotations were determined on a JASCO-DIP-181 digital polarimeter. MS were obtained under positive FAB conditions with JEOL DX-110 spectrometer. The ^{1}HNMR, ^{13}CNMR, 2D-NMR were measured with JEOL α-600 spectrometer. TLC was performed on RP-18 plates (E. Merck), using MeOH-H_2O (1:2 and 1:3). Spots were visualized under UV and by spraying with $FeCl_3$ solution.

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Plant material

Seeds of *Quercus robur*, were collected in July 1996 from the trees cultivated near Assiut University, Assiut, Egypt. A voucher specimen is deposited in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Assiut University. The plant is identified by Prof. Dr. Gamal Taha, Professor of Horticulture, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Extraction and fractionation

The seeds (1.9 kg) was finely milled and extracted with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure till syrupy consistence (250 g), and then subjected to fractionation with hexane (25 g), $CHCl_3$ (4.7 g), EtOAc (60 g) and n-butanol (30 g), successively.

Isolation

About 5g of the EtOAc soluble fraction was chromatographed on ODS column, using MP pump and MeOH-H_2O (1:2) to afford fractions, **I** (946 mg), **II** (377 mg), **III** (144 mg) and **IV** (618 mg) & MeOH-H_2O (1:1) afforded fractions **V** (730 mg) and **VI** (129 mg). Fr. **I** was rechromatographed on MCI-gel CHP-20P (20% MeOH-H_2O), to give gallic acid (850 mg). Repeated purification of each of fractions **II, III, V** and **VI** on ODS column and MCI-gel CHP-20P using MeOH-H_2O (1:2 & 1:1) yielded pure **1** (21 mg), **2** (29 mg), **3** (17 mg) and **4** (19 mg) respectively. Fraction **IV** was purified by rechromatography on ODS column to give methoxy gallic acid (300 mg).

1,2,3-tri-O-galloyl-ß-D-glucose (1):

Pale brown amorphous powder, $[\alpha]_D^{27}$ +47 (MeOH; c=302.4). Positive FAB-MS: m/z 637 ($C_{27}H_{24}O_{18}$) $[M+H]^+$. ^{1}HNMR (CD_3OD): δ 3.69 (1H, ddd, J= 2.2, 4.4, 9.5 Hz, H-5'), 3.80 (1H, dd, J= 4.4, 11.7 Hz, H-6'b), 3.87 (1H, t, J= 9.5 Hz , H-4'), 3.92 (1H, dd, J= 2.2, 11.7 Hz, H-6'a), 5.4 (1H, dd, J= 8.8, 9.5 Hz, H-2'), 5.52 (1H, t, J= 9.5 Hz, H-3'), 6.05 (1H, d, J= 8.8 Hz, H-1'), 6.91 , 7.02, 7.03 (each 2H, s, galloyl-H). ^{13}CNMR (CD_3OD): see Table 1.

1,2,3,6-tetra-O-galloyl-ß-D-glucose (2):

Pale brown amorphous powder, $[\alpha]_D^{27}$ +29.8 (MeOH; c=340.2). Positive FAB-MS: m/z 789 ($C_{34}H_{28}O_{22}$) $[M+H]^+$. ^{1}HNMR (CD_3OD): δ 3.96 (1H, t, J= 9.5 Hz, H-4'), 4.01 (1H, ddd, J= 2.2, 4.4, 9.5 Hz, H-5'), 4.52 (1H, dd, J= 4.4, 11.7 Hz, H-6'b), 4.61 (1H, dd, J= 2.2, 11.7 Hz, H-6'a), 5.44 (1H, dd, J= 8.8, 9.5 Hz, H-2'), 5.57 (1H, t, J= 9.5 Hz, H-3'), 6.09 (1H, d, J = 8.8 Hz, H-1'), 6.93, 7.02, 7.03, 7.12 (each 2H, s, galloyl-H). ^{13}CNMR (CD_3OD): see Table 1.

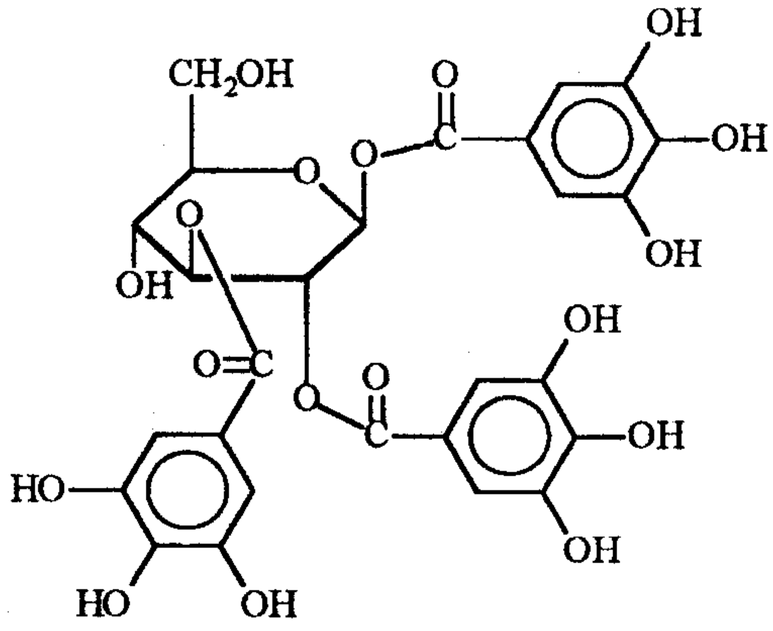
1,2,3,4,6-penta-O-galloyl-ß-D-glucose (3):

White amorphous powder, $[\alpha]_D^{29}$ +22.9 (MeOH; c=228.2). Positive FAB-MS: m/z 941 ($C_{41}H_{32}O_{26}$) $[M+H]^+$. ^{1}HNMR (CD_3OD): δ 4.38 (1H, dd, J= 4.4, 11.7 Hz, H- 6'b), 4.4 (1H, ddd, J= 2.2, 4.4, 9.5 Hz, H-5'), 4.51 (1H, dd, J= 2.2, 11.7 Hz, H-6'a), 5.58 (1H, t, J= 9.5 Hz, H-3'), 5.61 (1H, dd, J= 8.8, 9.5 Hz, H-2'), 5.89 (1H, t, J= 9.5 Hz , H-4'), 6.22 (1H, d, J= 8.8 Hz, H-1'), 6.89, 6.94, 6.97, 7.04, 7.1 (each 2H, s, galloyl-H). ^{13}CNMR (CD_3OD): see Table 1.

1,2,3,4,6-penta-O-galloyl-α-D-glucose (4):

White amorphous powder, $[\alpha]_D^{28}$ +118.1 (MeOH; c=226.8). Positive FAB-MS: m/z 941 ($C_{41}H_{32}O_{26}$) $[M+H]^+$. ^{1}HNMR (CD_3OD): δ 4.38 (1H, dd, J= 4.4, 11.7 Hz, H-6'b), 4.46 (1H, dd, J= 2.2, 11.7 Hz, H-6'a), 4.56 (1H, ddd, J= 2.2, 4.4, 9.5 Hz, H-5'), 5.49 (1H, dd, J= 3.7, 9.5 Hz, H-2'), 5.69 (1H, t, J= 9.5, H-4'), 6.11 (1H, t, J= 9.5 Hz, H-3'), 6.69 (1H, d, J= 3.7 Hz, H-1'), 6.92, 6.93, 6.99, 7.10, 7.20 (each 2H, s, galloyl-H). ^{13}CNMR (CD_3OD): see Table 1.

Gallic acid and methoxygallic acid were identified from their ^{1}HNMR data and direct comparison with authentic samples.



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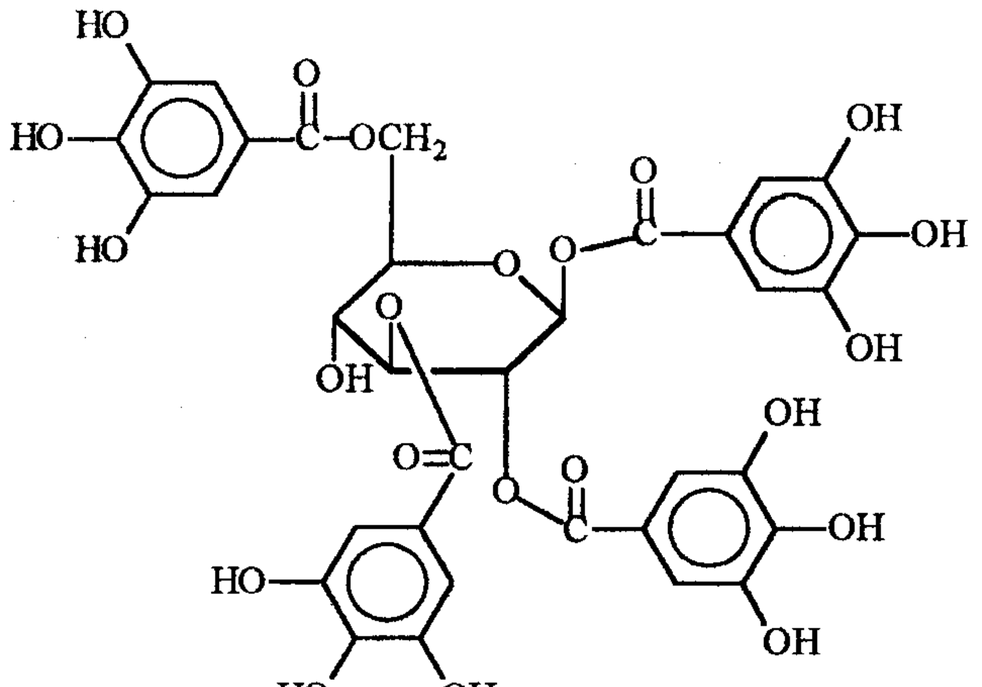

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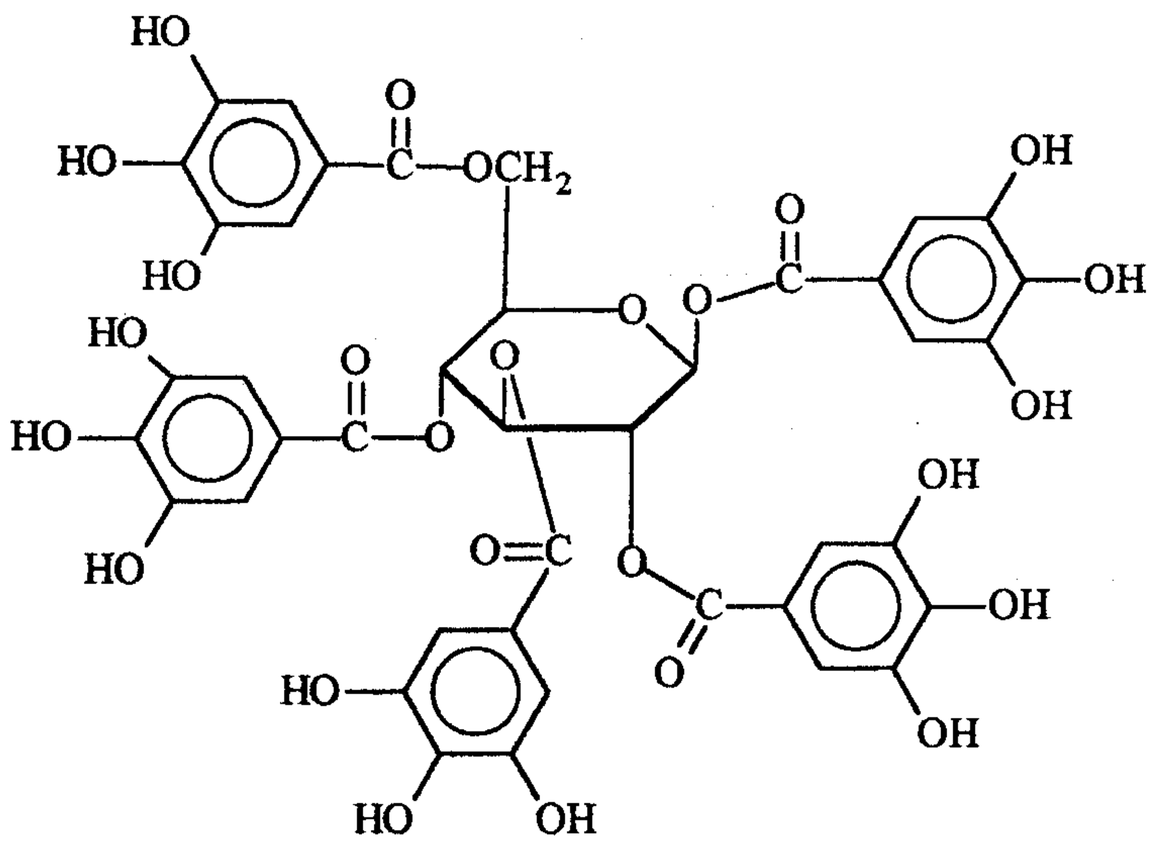

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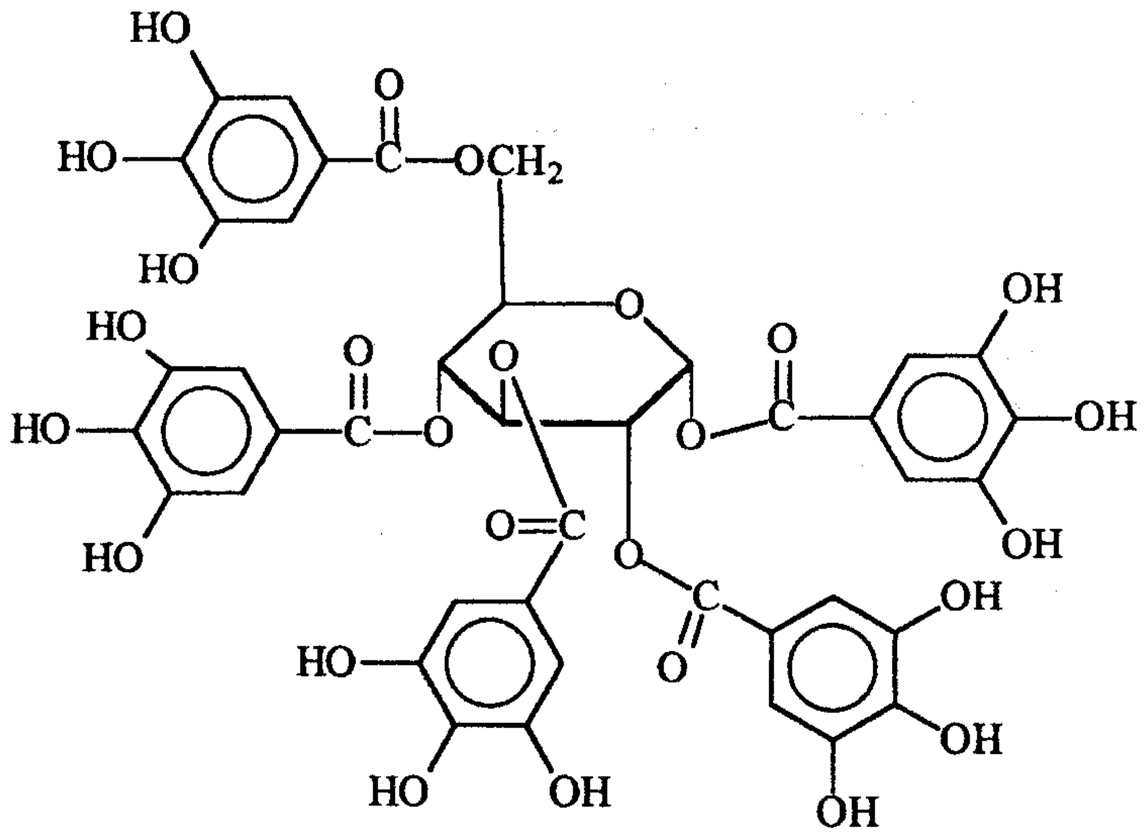

(4)

Table 1: ^{13}CNMR spectral data for compounds **1-4** in CD_3OD.

Group		1	2	3	4
Galloyl CO		166.39 167.16 167.78 --- ---	166.29 167.16 167.70 168.16 ---	166.22 166.93 167.01 167.29 167.93	165.96 166.88 167.09 167.61 167.92
Galloyl C-1		119.96 120.47 121.04 --- ---	119.83 120.39 120.96 121.21 ---	119.73 120.22 120.25 120.37 121.06	120.00 120.18 120.23 120.42 121.08
Galloyl C-2,6		110.34 110.39 110.52 --- ---	110.21 110.36 110.39 110.57 ---	110.36 110.39 110.42 110.47 110.64	110.33 110.41 110.48 110.51 110.55
Galloyl C-3,5		146.30 146.33 146.49 --- ---	146.30 146.48 --- --- ---	146.28 146.36 146.43 146.46 146.54	146.30 146.35 146.45 146.49 146.78
Galloyl C-4		139.93 140.13 140.61 --- ---	139.9 140.2 140.7 --- ---	140.02 140.13 140.31 140.36 140.77	140.02 140.19 140.37 140.40 140.79
Sugar moiety	C-1' C-2' C-3' C-4' C-5' C-6'	93.87 72.39 76.74 69.30 78.99 61.84	93.88 72.35 76.44 69.62 76.60 63.96	93.83 72.20 74.12 69.81 74.42 63.12	90.98 71.62 71.62 69.70 72.00 63.07

RESULTS AND DISCUSSION

Repeated ODS column chromatography and further purification on MCI-gel CHP-20P of the EtOAc soluble fraction of the MeOH extract of Q. robur seeds, afforded compounds **1-4**. All the isolated compounds gave dark blue colour with $FeCl_3$.

Compound **1**, showed a prominent $[M+H]^+$ ion peak at m/z 637 in the positive mode FAB-MS, consistent with the molecular formula $C_{27}H_{24}O_{18}$. Its ^{1}HNMR spectrum showed the presence of three galloyl groups at δ 6.9, 7.02 and 7.03 (each 2H, s) and an anomeric proton at δ 6.05 (1H, d, J= 8.8 Hz). Moreover, the ^{13}CNMR spectrum confirmed the presence of

three galloyl groups and one glucose moiety, from the consideration of the sugar carbon chemical shifts as detailed by Nishizawa and co-workers.[17,18] The configuration at the glucose C-1 position was concluded to be ß on the basis of the J-value (8.8 Hz) of the anomeric proton signal at δ 6.1. The location of the galloyl groups was concluded to be at C-1', C-2' and C-3' positions of the glucose moiety on the basis of the significantly downfield shifts of H-1' (δ 6.1), H-2' (δ 5.4) and H-3' (δ 5.5). The spectral data and HMBC spectrum are in accordance with the reported data for 1,2,3-tri-O-galloyl-ß-D-glucose.[19-21]

To the best of our knowledge, this is the first report on isolation of compound **1** from plants of the family *Fagaceae*.

The ^{1}HNMR spectrum of compound **2**, displayed 4 galloyl groups and one glucose moiety, consistent with the observation of $[M+H]^+$ ion peak at m/z 789 ($C_{34}H_{28}O_{22}$) in the FAB-MS. The downfield shift of H-6' (a,b) (δ 4.61, 4.51) and C-6'(δ 64.0) in the ^{1}HNMR and ^{13}CNMR spectra respectively in comparison with compound **1**, thus confirming that there is an additional galloyl group, which must be at C-6' position of the glucose moiety.[9] Thus, compound **2**, should be 1,2,3,6-tetra-O-galloyl-ß-D-glucose. The spectral data of this compound are similar to those reported from *Q. infectoria*.[22]

The FAB-MS of compounds **3** and **4**, exhibited the same $[M+H]^+$ ion peak at m/z 941 ($C_{41}H_{32}O_{26}$), consistent with the ^{1}HNMR and ^{13}CNMR data, which suggest the occurrence of a glucose moiety and 5 galloyl groups in both compounds. In addition, ^{1}HNMR spectrum explained the mode of glucosidic linkage to be -ß- in compound **3**, based on the large coupling constant of the anomeric proton at δ 6.22 (d, J= 8.8 Hz), while the small one (J= 3.7 Hz) of the anomeric proton at δ 6.69 in compound **4** showed the anomeric center to have α-configuration. Comparison of the ^{13}C-chemical shift (δ 90.98) of the C-1 atom in **4** with that (δ 93.83) in the ß-anomer of **3** also confirmed the mode of the linkage to be α. The location of the galloyl groups in both compounds, was concluded to be at C-1', C-2', C-3', C-4' and C-6' of the glucose moiety on the basis of the significantly downfield shifts of H-1', H-2', H-3', H-4' and H-6' (a,b). Thus compounds **3** and **4** were identified as 1,2,3,4,6-penta-O-galloyl-ß-D-glucose and 1,2,3,4,6-penta-O-galloyl-α-D-glucose respectively. Compound **3** was reported from *Q. infectoria*[22] while compound **4** was reported only from the rhizomes of *Nuphar japonicum* family *Nymphaeaceae*[23] and is reported here for the first time in family *Fagaceae*.

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