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BIOLOGICALLY ACTIVE ABIETANE DITERPENES FROM *TAXODIUM DISTICHUM* SEEDS

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تم في هذا البحث دراسة محتوى الخلاصة الكلوروفورمية لبذور نبات التاكسوديم ديستيكم حيث تم فصل أربع مركبات من مشتقات الدايتيربين وهي تاكسودون ، تاكسوديون ، ١١-هيدروكسي مونتبريتول و فيروجينول. وقد تم التعرف علي المركبات المفصولة باستخدام الطرق الطيفية الحديثة وقد تم فصل المركب الأول والثاني من قبل من نفس النبات و ثبت أن لهما تأثيرا مثبطا ضد الخلايا السرطانية. وقد ثبت لنا من خلال إجراء التجارب علي المركبات الأربع أن التاكسوديون له تأثير مثبط ضد إنزيم البروتييز لفيروس الأيدز.

Four diterpenes, taxodone, taxodione, 11-hydroxy montbretol and ferruginol (1-4) respectively, were isolated from Taxodium distichum (L.) Rich seeds. The first two compounds 1 and 2, were isolated before from the same source and has been reported to possess antitumor activity. Compounds 3 and 4 were isolated for the first time here from the genus taxodium. The structures of the isolated compounds has been determined through intensive spectral analysis. On the other hand HIV-1 PR inhibitory activity for the four compounds has been evaluated where, only compound 2, showed a potent effect in 0.1 mM concentration.

INTRODUTION

Taxodium distichum (L) Rich, Family Taxodiaceae is a big tree attaining a height of 40 meters, known in America as Bald Cypress or Swamp Cypress, native from Mississippi to Florida in swampy regions and along rivers. It has been introduced to Assiut since 1957.[1] The unisexual flowers are carried on the same plant, the males in pendulous, branching clusters, the females in little strobiles. The wood of the trunk is especially valued for its excellent resistance to decay and termites.[1-2]

In our course of study for natural metabolites in woody plants, which might be useful as HIV-1 PR inhibitors, we have studied the components of *Taxodium distichum* commonly found in Assiut University campus. Four diterpenes could be identified and isolated from the chloroform-soluble extract of the seeds.

Previous reports on the chemical composition of the same plant, revealed the presence of biflavonoids and abietane diterpenes.[3-5] Taxodone and taxodione were reported to have significant *in vivo* activity against Walker intramuscular carcinosarcoma 256 in rats and *in vitro* activity against cells derived from human carcinoma of nasopharynx (KB).[5]

EXPERIMENTAL

^{1}H and ^{13}CNMR ($CDCl_3$) were measured on Varian JMNGX 500 spectrometer (500 MHz for ^{1}HNMR and 125 MHz for ^{13}CNMR) with TMS as int. standard, UV, UV\VIS Shimadzu 2200 instrument (Shimadzu Corporation, Kyoto, Japan), IR, IR impact 410 FTIR spectrometer, MS at 70 ev (JEOL JMS-DX 300L Mass spectrometer for measurement of EI), silica gel 60 F_{254} (E. Merck) for TLC. Plant material was collected from Assiut University campus, in June 1994.

Extraction and isolation of the diterpenoids

Dried and powdered *T. distichum* seeds (120 g) was extracted with methanol and the solvent removed under reduced pressure giving

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an extract (8 g) which was partitioned between chloroform and water. The concentrated organic phase (5 g) was chromatographed successively on silica gel eluted with mixtures of n-hexane-EtOAc of increasing polarity. Fractions were collected. Repeated chromatography on sephadex LH-20 eluted with methanol and MPLC si gel eluted with chloroform-hexane for these fractions gave the following compounds taxodione (1) (8 mg), taxodone (2) (5 mg). 11-hydroxy montbretol (3) (6 mg) and ferruginol (4) (5 mg) (Fig. 1).

Taxodione (1) showed λ_{Max}^{MeOH} 320, 332, 440, IR (KBr) 3450, 1650, 1635, 1600 and 910 cm^{-1}. ^1H and $^{13}CNMR$ ($CDCl_3$) see Tables (1 and 2).

Taxodone (2) showed λ_{Max}^{MeOH} 316, IR (KBr) 3690, 3350, 1630, 1570 and 915 cm^{-1}. ^1H and $^{13}CNMR$ ($CDCl_3$) see Tables (1 and 2).

6,11,12-trihydroxy-5,8,11,13-abietatetra-en-7-one (11-hydroxy montbretol) (3), IR (KBr) 3430, 3390, 3030, 1630, 1585 and 1555 cm^{-1}. ^1H and $^{13}CNMR$ ($CDCl_3$) see Tables (1 and 2).

Ferruginol (4) IR (KBr) 3430 and 3390 cm^{-1}. ^1H and $^{13}CNMR$ ($CDCl_3$) see Tables (1 and 2).

By carrying out HIV-1 PR inhibitory activity test[11] for the isolated compounds, compound 2 was found to have the highest activity (83.3 ± 5.2, in 0.1 mM). This result encourages us to plan for more detailed study for this compound in the future.

Table 1: ^1HNMR data of compounds 1-4 (500 MHz, $CDCl_3$)

position	1	2	3	4
1a, b	1.74, 2.95, m	2.91, m	1.75, 2.95, m	1.38, 2.16, m
2a, b	1.60, 1.70, m	1.6, m	1.90, 1.71, m	1.59, 1.74, m
3a, b	1.22, 1.41, m	1.20, m	2.03, 1.43, m	1.20, 1.46, m
5	2.60, s		---	1.31
6a, b	---	4.69, m	---	1.68, 1.85, m
7a, b	6.21, s	6.55, d (2.8)	---	2.76, 2.87, m
11	7.60 (OH)	7.49 (OH)	---	6.6, s
14	6.88, s	6.81, s	7.72, s	6.85, s
15	3.06, septet (6.8)	3.06, septet (6.8)	3.05, septet (6.8)	3.1, septet (6.9)
16	1.16, d (6.8)	1.16, d (6.8)	1.29, d (6.8)	1.22, d (6.9)
17	1.18, d (6.8)	1.18, d (6.8)	1.32, d (6.8)	1.23, d (6.9)
18	1.11, s[a]	1.11, s	1.45, s	1.16, s
19	1.27, s[b]	1.17, s	1.46, s	0.91, s
20	1.27, s[c]	1.22, s	1.68, s	0.94, s

[a,b,c]= Signals are interchangeable ( )= J value in Hz

Table 2: ^{13}CNMR data of compounds 1-4 (125 MHz, $CDCl_3$)

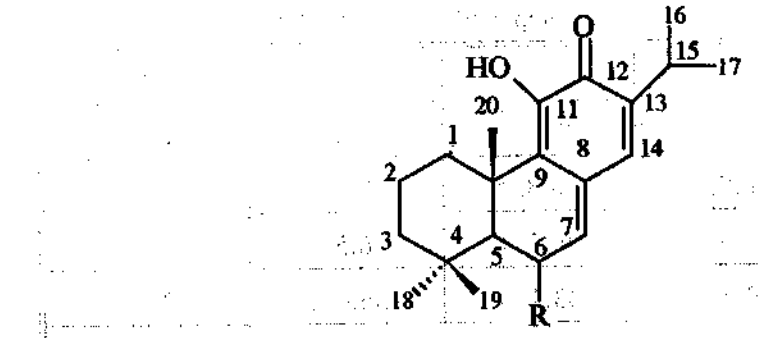
Position	1	2	3	4
1	36.9, t	37.6, t	30.4, t	38.8, t
2	18.5, t	18.8, t	17.9, t	19.3, t
3	42.5, t	40.7, t	36.5, t	41.7, t
4	29.6, s	43.1, s	36.4, s	33.4, s
5	62.9, d	58.0, d	143.2, s	50.3, d
6	201.1, s	70.1, d	142.9, s	19.2, t
7	136.1, d	135.7, d	179.9, s	29.7, t
8	139.9, s	143.4, s	120.9, s	127.2, s
9	144.9, s	126.2, s	138.2, s	148.6, s
10	33.2, s	37.6, s	40.7, s	37.5, s
11	145.3, s	141.9, s	140.8, s	110.9, d
12	181.7, s	181.7, s	145.3, s	150.6, s
13	125.5, s	130.4, s	132.6, s	131.3, s
14	133.9, d	149.1, d	116.4, s	126.6, d
15	27.1, d	26.7, d	27.1, d	26.8, d
16	21.2, q	21.7, q	22.4, q	22.6, q
17	21.6, q	21.4, q	22.6, q	22.7, q
18	32.2, q	34.1, q	27.9, q	24.8, q
19	21.8, q	22.8, q	27.4, q	21.6, q
20	21.1, q	20.8, q	27.95, q	33.29, q

RESULTS AND DISCUSSION

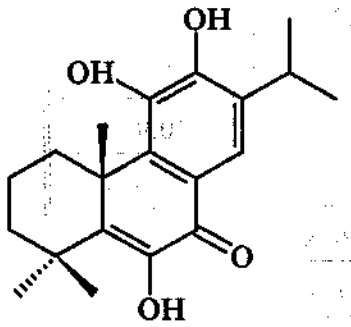
The UV spectrum of compound **1** in methanol, suggested the presence of quinonoid structure and its IR spectrum supported our expectation by the presence of absorption bands at 1650 and 1635 cm^{-1}, in addition to the significant absorption bands for hydroxyl group at 3450 cm^{-1} and double bonds at 910 cm^{-1}. The ^{1}HNMR spectrum of this compound clearly indicated the presence of an isoprpoyl function on aromatic ring as two methyl proton doublets at δ 1.16 and δ 1.18 and one proton septet at δ 3.06 (J = 6.8 Hz), and one angular methyl group at δ 1.27. The mass spectrum of compound **1** diplayed molecular ion peak $(M)^+$ at m\z 314, calculated for $C_{20}H_{26}O_3$.

Matching the data obtained for compound **1** with those reported in the literature[5] for taxodione, confirmed identidty.

The ^{1}HNMR spectrum of compound **2** was very similar to compound **1** but it clearly displayed the presence of one ß-H at δ 4.69 suggestion the presence of another OH function.



1, R= O
2, R= OH



3

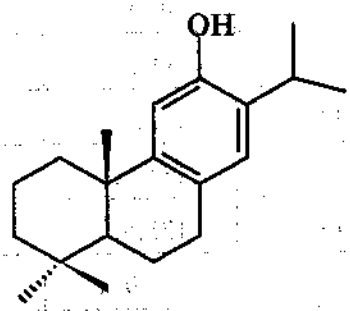


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Fig. 1: The diterpenes isolated from the seeds of *T. distichum*

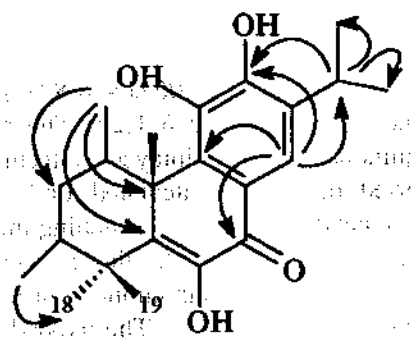

Fig. 2: HMBC of compound **3**

The mass spectrum of compound 2 displayed molecular ion peak M^+ at m/z, 316 calculated for $C_{20}H_{28}O_3$.

The difference in the IR and mass spectra of the two compounds suggested the replacement of a keto function in compound 1 with a hydroxyl group at C-6 in compound 2. Inspection of the compounds isolated previously from the same plant, revealed that compound 2 should be taxodone.

The ^{1}HMNR spectrum of compound 3 was very similar to montbretol isolated before from *Salvia montbretti*,[6] except for the absence of a signal at δ 6.45 of the aromatic proton on C-11. Moreover the ^{13}CNMR showed a strong downfield shift for C-11 than that in montbretol (from δ 108.70 to δ 140.8). Indicated the presence of an oxygen function on this carbon which is displayed by an OH band in the IR spectrum at 3380 cm^{-1}. Matching the IR, ^{1}HNMR and ^{13}CNMR data of compound 3 with other compounds in the literature revealed that this compound has the same structure of 6,11,12-trihydroxy-5,8,11,13-abietatetra-en-7-one a compound isolated from *salvia phlomoides*.[7] Final proof that compound 3 has the structure depicted in formula 3 was obtained by carrying out ^{1}H-^{1}H and ^{1}H-^{13}C COSY as well as HMBC experiment (Fig. 2). This compound has not been isolated before from the genus Taxodium.

Compound 4, showed absorption bands at 3590 and 3380 cm^{-1} in its IR spectrum, indicating the presence of phenolic OH and unsaturated carbon bonds at 910 cm^{-1}. The ^{1}HNMR spectrum of compound 4 (Table 1) showed signals of an isopropyl group and three methyl groups identical with those found in the other Taxodium diterpenes. The mass spectrum of this compound revealed molecular ion peak M^+ at m\z 286 calculated for $C_{20}H_{30}O$. This spectrum was similar to that published for ferruginol.[7]

This compound has been identified as ferruginol, a diterpene phenol isolated before from families Labiateae, Podocarpaceae, Cupressaceae and also from family Taxodiaceae (*Cryptomeria japonica* D.Don.).[6,8-10]

Here we report, for the first time, the ^{1}H and ^{13}CNMR data of ferruginol (not its acetate) (Tables 1 & 2).

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