Chemical Constituents of Artemisia Species Occurring in Pakistan

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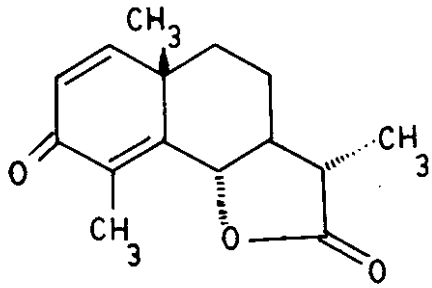
Summary: Artemisia (Family Compositae) is a large genus of small herbs and its 280 species are found in Northern Hemisphere in arid regions [1]. About thirty species of Artemisia have been reported to grow naturally in Pakistan [2]. Some work on chemical constituents of these plants has been reported, however, no research work seems to have been done on nineteen of these species (see table 1).

Artemisias are bitter aromatic herbs or low shrubs often with much divided leaves and inconspicuous flowers borne on numerous small heads. These herbs are of economic importance to Pakistan as some of these are of medicinal value and yield essential oils while few are useful as fodder [1]. Some of these species contain santonin, an anthelmintic drug [3]. Several santonin containing species of Artemisia like A.maritima Linn., A.brevifolia Wall, A.herba-alba Asso, A.kurramensis Qazilbash occur in Pakistan [2,4,5] but these different species appear to be only varieties of A.maritima L. instead of being separate species [2,4]. Artemisia maritima L. is found in Kurram valley, Gilgit Agency, Kaghan valley, Chitral, Dir, Swat, Khyber Agency, Indus Kohistan, Waziristan and large barren areas of Baluchistan [4]. Santonin (1) is extracted from Artemisia mainly collected near Parachinar in Kurram valley [6].

A new oral hypoglycaemic agent, vulgarin (2), has been reported [7] as the main constituent of A.vulgaris L. which appears to possess important medicinal properties. Its leaves are given in Indian medicine for nervous and spasmodic affections and it is said to be antiseptic, expectorant and anthelmintic [8]. Tincture of A.absinthium L. is used as tonic and digestive and dried leaves and flowering tops of the plant have medicinal properties [9].

Ethereal oils of A.scoparia Waldst. and Kit. are raw materials for the drug, "artemisol", which is used in the therapy of urolithiasis [10]. Dried young plants of A.scoparia Waldst. and Kit. are used in "Ing-Chen Hao", a Chinese drug for treatment of jaundice [11]. Artemisia annua L., a Chinese traditional herb, contains the antimalarial constituent, "quinghaosu" [12]. The extracts of A.japonica Thunb., are antifungal [13]. These species of Artemisia also occur in Pakistan.

The main use of Artemisia plants particularly A.maritima L in Pakistan has been for the extraction of 'santonin', an anthelmintic drug of choice for human and veterinary use. Lately, export of this drug has considerably decreased on account of competition from much cheaper and



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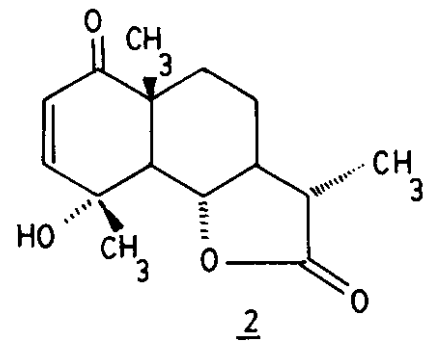

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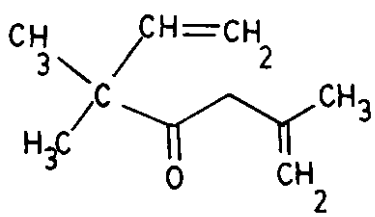

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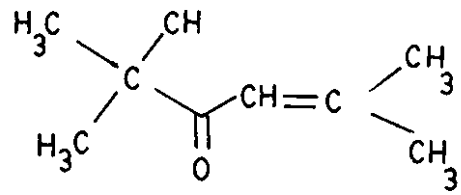

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equally effective synthetic anthelmintics. It is therefore, necessary to find alternative or new uses to keep the collection of these herbs profitable for the people of our Northern areas. With this view, the following review of literature was undertaken to study the chemical constituents of various Artemisia species occurring in Pakistan, and to assess the potential uses of these plants and their constituents. Table-1 shows these species and their main chemical constituents. The review of the work is of the Artemisia plants occurring in Pakistan only, or of the same species growing elsewhere in the world and reported from those sources. It is, however, well realized that the type or quantity and relative proportion of different compounds in the same species growing in Pakistan or elsewhere in the world may vary considerably on account of differences in climatic, agronomic condition, and stage of its growth at the time of collection.

Essential Oils

The presence of essential oils appears to be a characteristic of these herbs. The dried A.absinthium L. contains 0.5% essential oil with the highest amount in June-July crop [14]. The gas chromtograph of the essential oil from A.absinthium L. shows the presence of α-thujone, α-pinene, camphene, ρ-cymene, 1,8-cineole, methyl heptanone, β-phelandrene, caryophyllene oxide, α-terpineol, thujyl alcohol, geraniol, thujyl acetate, caryophyllene, α-himachalene, α-cadinene and elemol [15]. Many of these are very valuable components in perfumery industry.

Lauric, myristic, palmitic, stearic, oleic, linoleic and arachidic acids were identified in petroleum ether extracts of A.absinthium [16]. Palmitic (34%), arachidic (26.2%) and linoleic (27.5%) acids were the major fatty acids [16]. The volatile oil of the leaves (0.22%), flowers (0.35%), stems (traces) and

Table-1: Occurrence and main chemical constituents of various Artemisia species occurring in Pakistan.

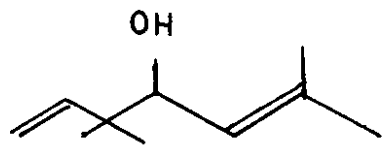
S.No.	Species of Artemisia	Main Chemical Constituents	Area of Occurrence in Pakistan
1.	A.absinthium L.	α and β-thujones [17] artabsin (26) [45-47] artemolin (27) [48] anabsin (23) [49,51] anabsinthin (27) [49,50] absinthin (30) [46,47,52,53] sesartemin (54) [83]	Kurram, Shalizon, Ali-Khel, Parachinar, Poonch and Kashmir
2.	A.amygdalina Deric.		Kagan valley, Kashmir
3.	A.annua L.	Artemisia (3) and iso-artemisia (4) Ketones [18], arteannuin B (18) [43], quinghaosu (19) [44,12] flavanol (38)	Peshawar to Waziristan at 5500'.
4.	A.biennis Willd	-	Ladak, Kashmir-Ladak Road.
5.	A.Compbelli H & T Ex Charke	-	Kashmir
6.	A.desertorum Spreng (A.Jacquemontiana Bess)	-	Astor (Das Kirrim)
7.	A.dracunculus L.	Essential Oil [8]	Mt.Tilla,Salt Range Jhelum in Punjab; Chitral, Gilgit,Balti, Asotr,Nobra and Shyok valley; at 3-5,000 m.
8.	A.Grata Wall (syn.A.Hypoleuca Edgew. A.myrriantha Pamp.)	-	Kagan Valley,Change and Danga Galis,Murree Hills, Ghora Gali, Poonch,Kashmir
9.	A.herba-alba Asso (Syn.A.maritima L., A.compestris auct. non L)	Santolina alcohol [22] α-santonin (1) [35] torrentin (15) [38] Herbolide (A,B,C) [32,34] flavone (39) [65].	Common in Baluchi Deserts, Quetta Sarrange, Hanna Valley
10.	A.laciniata Willd.	-	Chitral (Kala Drosh) Gilgit,Baltit, Hunza, Swat (W.of Kalam) Islamabad; at 1600-4,000 m.

S.No.	Species of Artemisia	Main Chemical Constituents	Area of Occurrence in Pakistan
11.	A.marcocephala Jacq.ex Bess (syn.A.griffithiana Boiss)	Chamazulene (9) [25]	Chitral, Ladak.
12.	A.maritima L. (syn.A.Kurramensis Qazilbash, A.aucheri Boiss., A.brevifolia Wall.	α-santonin (1) [27,34-37] cineole [8],thujone [8,25,28], camphor [8],	Baluchistan, Kurram, Chitral,Swat,Astor, Gilgit, Balti, Nobra, Ladak,Upper Hunza, Kashmir at 7-14,000'.
13.	A.minor Jaeq.ex.Bess (syn.A.tibetica H & T.; A.sieversiana var.tibetica Clarke).	-	Balti, Ladakh at 4,000-5,800 m.
14.	A.macrocraftiana Wall ex Dc. (syn.A.hypoleuca H & T)	-	Kurram, Ladak,Kashmir.
15.	A.paniculata HK.f.,l.c.	-	Balti.,Ladak at 3000-5,500 m.
16.	A.parviflora Roxb. (syn.A.japonica Thunb.var.parviflora (Roxb)Pamp.)	Scoparone (400) [73,74] Capillarin (43) [73]	Kurram,Kurram-Habib-kala plains,Dir,Chitral Lowari pass, Swat (Common in Utror-kalam area), Kagan Valley, Changla Gali, Astor,Rothu and Rupal Nullah, Ladak, Murree,Poonch,Kashmir.
17.	A.persica Boiss.		Kurram(Sergal,Shokor Shal),Chitral (Drosh), Swat (Kalam, Gabral), Ladak,Balti,Kashmir, Kagan Valley; at 5-14,000'.
18.	A.purpurascens Jacq. ex-Besser.	-	Kurram Valley,Kashmir.
19.	A.rutifolia Steph. ex.Spreng.	-	Kurram (huriab Dt.) Gilgit at 3-5000 m.
20.	A.sacrorum Ledeb.	Cineol [8],Camphor [8]	Chitral (Drosh,Shishi) Swat,Kalam,Balti., Gilgit,Kashmir at 3,000 to 5,500 m.
21.	A.salsoloides Willd	-	Parang Valley

S.No.	Species of Artemisia	Main Chemical Constituents	Area of Occurrence in Pakistan
22.	A.scoparia Waldst. and Kit. (A.capillaris Thunb. var.scoparia (W&K) Pamp.)	rutin [65],quercetin-3-glucogalactoside [45] scoparone [66-69]	Malir (Sind),Las Bela (baluch.),Ziarat, Kurram (baluch.),Ziarat,Kurram (Horiab Dt.),Peshawar, Lahore,Salt Range(Punjab) Dir,Chitral,Lower Swat (Saidu),Gilgit,Astor, Balti.,Kashmir,plains to 4,000 m.
23.	A.seiversiana Ehrh.ex Will (syn.A.pallens Wall. teste ext.Pamp).	Essential oil [8,28] Artabsin (26) [54] Absinthin (30) [54] sesquiterpenoid (44) [78] (49) [80] davanafurans (45-48) [79].	Chitral (Lut Kho), Balti., (Shardu) Gilgit.
24.	A.simplicifolia Pamp.	-	Karakorum mountains.
25.	A.stracheyi H & T. ex Clarke.	-	Rupshu (Pugha); 4,700-5,700 m.
26.	A.stricta Edgew.	-	Ziarat (Baluch.)Satpura (Balti.,); 2,700-4,500 m.
27.	A.tournefortiana Reichb.	-	Ziarat,Harboi (Baluchi.), Kurram (Shalizon and Hariab Dts.),Chitral (Drosh),Swat (Mt.Ilam), Gilgit (Shykok Valley and Nubra), Ladakh (Nurla)Nathia, Dunga and Changla Galis,Common in Kashmir,at 2,000-3,800 m.
28.	A.vestita Wall.ex DC. (too close to A.laciniata and A.sacrorum).	-	Trans Indus,near Peshawar, Salts range Swat (Mingora), Kashmir, at 1700-3,000 m.
29.	A.vulgaris L. (syn.A.indica Willd. A.elegantissima Pamp., A.dubia Wall., A.roxburghiana)Wall.ex Besser	Cineol [31,32] thujone [31] vulgerol (13) [33], vulgarin (2) [7] hydromatricaria ester [82]	According to Pampanini, there is not true A.vulgaris in the region and he destributes this specimen referred

S.No.	Species of Artemisia	Main Chemical Constituents	Area of Occurrence in Pakistan
			Hooker to A.dubia, incisa, elegantissima while Dr. Jarett at Kew refers it to A.indica occurring in temperate zone from Peshawar, Chitral,Swat,Hazara, eastward.
30.	A.wellbyi Hemsl. and Pearson.	-	Rupshu (Hunle-Da), at 15,000.

Balti. stand for Baltistan, Baluch. for Baluchistan. -- no study available.



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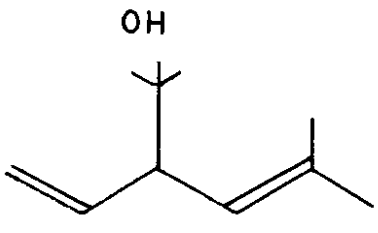

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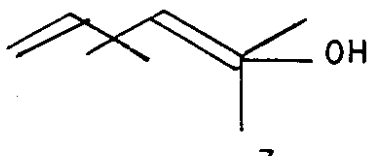

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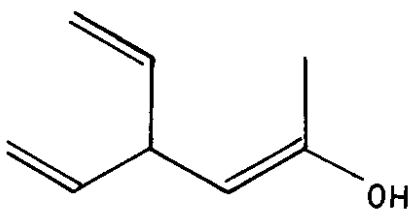

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herb (0.3%), was analysed by thin layer and gas chromatography and α-pinene, β-pinene, p-cymene, β-phellandrene, azulene, cineole, thujone, nerol, neryl acetate and cadinene were found in it [16]. The main components [17] of the essential oil of A.absinthium L. appear to be α and β-thujone and thujyl alcohol, free or combined as acetate, palmitate and isovalerate. A commercial oil is produced in the U.S.A. from A.absinthium [8] L. for perfumery industry.

The essential oil of Artemisia annua L. is yellow in colour with a refreshing, slightly balsamic odour resembling that of basil. The highest amount of oil is found in the tops just at the time of the appearance of most of the flower buds and open flowers.

Distillation for oil should be carried out on fresh plants. It contains [18] α-pinene, camphene, cineols, cadinene, caryophyllene, some sesquiterpene alcohols, artemisia-ketone (3) and isoartemisia-ketone [19,20] (4), acetic acid, butyric acid, cuminaldehyde and phenol [18]. Recently, camphor, β-caryophyllene and β-pinene have been identified in the volatile oil of A.annua L. in addition to camphene and isoartemisia ketone [21].

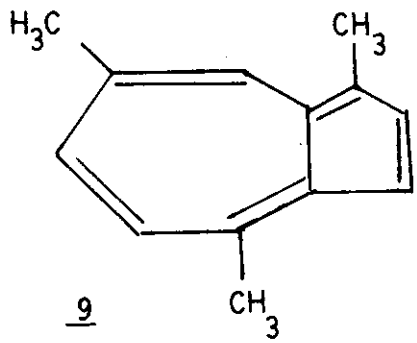
Artemisia species, A.drancuculus L., is cultivated in France for its precious essential oil which is called oil of "taragon". This herb contains 0.3% essential oil with anise-like smell. The leaves and essential oil from this herb are used for flavouring and as a spice [8]. This species also occurs in Pakistan in the Punjab, Chitral, Gilgit and Baltistan [2].

The antibacterial activity [22] of whole plant extract of A.herba-alba Asso is concentrated in the essential oil fraction. The essential oil was fractionated by column chromatography and these fractions were tested for antibacterial activity. The principle component of the most active fraction was santolina alcohol (6) [22]. Four non-head to - tail monoterpene alcohols, comprising 30% of the oil, were also identified in the essential oil from A.herba-alba growing in Negev desert. These irregular monoterpene alcohols, relatively rare in nature, were artemisia alcohol (5), santolina alcohol (6), Yomogi alcohol (7) and lyratal (8) [23]. The essential oil of A.herba-alba Asso from Morocco contained α-pinene, camphene, β-pinene, sabinene, p-cymene, 1,8-cineole, α and β-thujone, camphor, myrtenal, cuminaldehyde, l-terpinene-4-ol, trans-pinocarveol, borneol, myrtenol, bornyl acetate and probably β-guaiene and α-elemene. The three Ketones, α and β-thujone and camphor represented 60-80% of the total essential oil [24].

Aqueous extraction and distillation of A.macrocephala Jacq.ex Boss occurring in Altai mountains of Russia [25] (and also in Ladak and Chitral in Pakistan) [2], gives an oil in the range of 0.15 to 0.48% of air dried raw material depending on the growth phase of the plant [25]. Acetic, propionic, enanthic acids, o-cresol, p-cresol, α and β-pinenes, Δ^3-carene, limonene, 1,8-cineole, p-cymol, thujone, camphor, borneol and chamazulene have been identified and chamazulene [25] is of special practical value in this oil. The highest amount of chamazulene was found in the inflorescences in the blooming and fruit-set period and in the leaves during the budding phase. A preliminary 2 hours treatment of the raw material with alkali and subsequent acidification with 5% solution of sulphuric acid increased content of chamazulene (9) which is an anti-inflammatory and antipyretic agent [26].

The content of essential oil in the domestic A.kurramensis Qazilbash (collected in September 1958) was 0.618% in the flower buds and leaves and 0.585% in the whole herb. It contained α-pinene (2.76%), cineole (20.09%), β-thujone (62.74%), camphor (10.85%), α-terpineol (0.89%), β-pinene, and an unidentified low boiling fraction [27]. Another report considered Pakistani A.kurramensis to be important due to the composition of its essential oil (1.23%) containing thujone (33.86%) and thujyl alcohol (19%) [28].

Essential oil of A.pallens Wall (the same as A.siever siana Ehrh.ex Willd.



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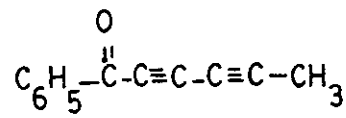

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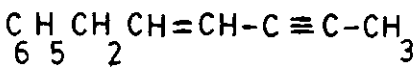

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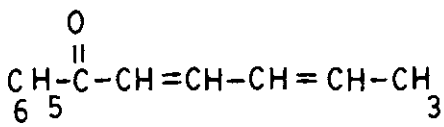

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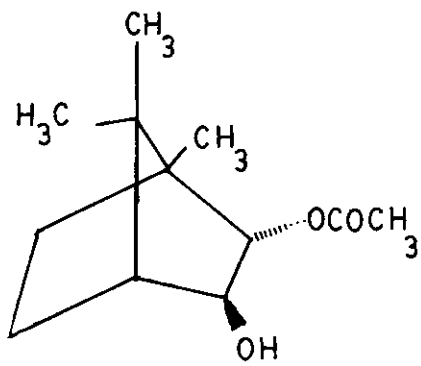

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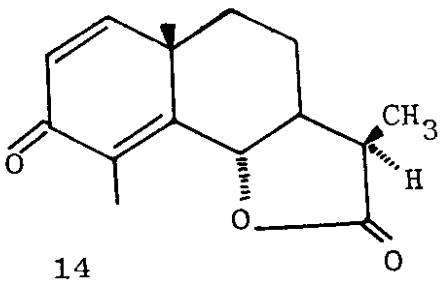
in Pakistan) contains camphene, p-cymene, sabinen, γ-terpinene, 1,8-cineole, isoborneol, geraniol, borneol, eugenol, methyl eugenol, methylisoeugenol, acetoeugenol, γ-cadinene, farnesol and linalool [29]. Oil of A.pallents Wall is very popular in perfumery in the U.S.A. and the yield of essential oil from this plant is in the range of 0.22-0.58% [8].

The essential oil of A.capillaris Thunb. (cf. A.scoparia Waldst. and Kit. [2] in Pakistan) contains β-pinene, scopoletin, esculetin-6,7-dimethyl ether, capillin (10), capillene (11) and capillone (12) [30].

A thick yellow commercial oil, a by-product of santonin manufacture, is obtained from A.maritima L. It varies both in quantity (2-3%) and composition. The essential oil from the corresponding Turkistan variety has been found to contain cineole (27.8%), and thujone (7-8%), while A.maritima L.var.Kadzkewicz yields an oil (0.6%) which contains 36% camphor [8].

The herb A.sacrorum Ledeb. yields 1% of an essential oil containing cineole (19-26%) and camphor (6%). Fractional distillation of the oil from the leaves of A.vulgaris L. var.



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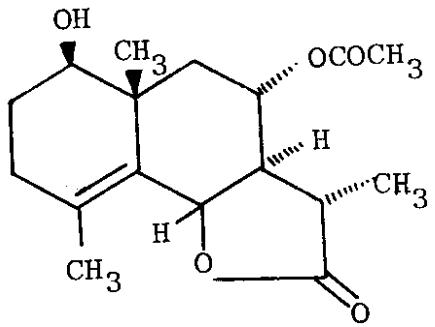

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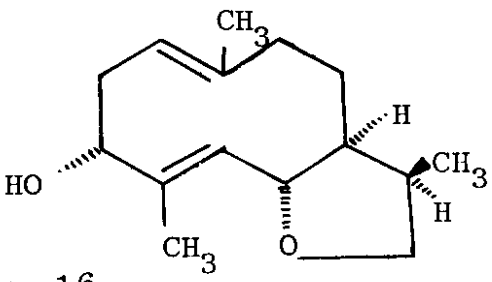

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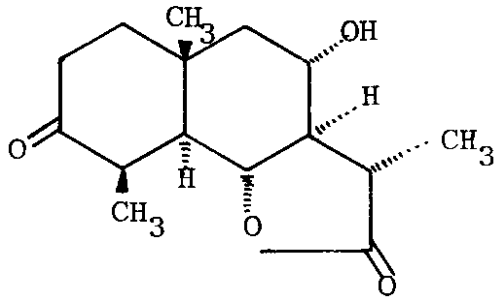

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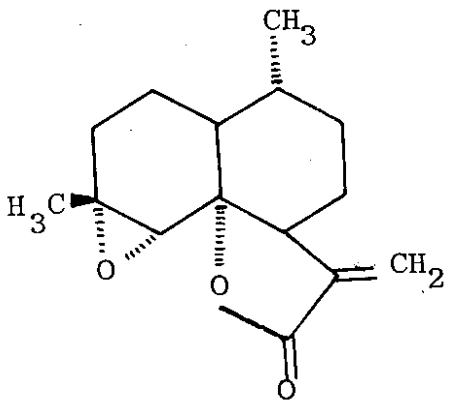

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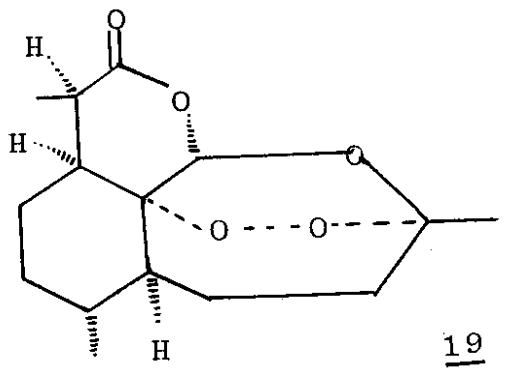

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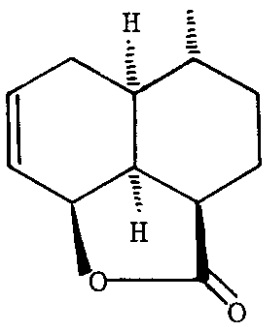

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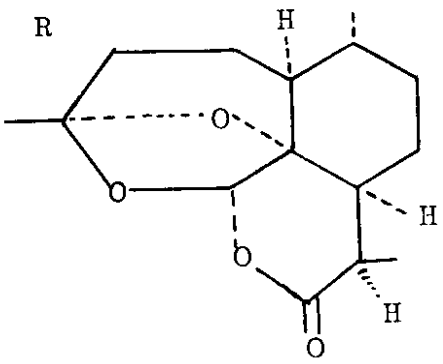


(21) R = H

(22) R = OH

indica Maxim showed that more than 50% of oil is cineole [31,32] and the rest is a sesquiterpene alcohol and its ester and β-thujone [31]. Vulgerol (2-endo-acetoxy-3-exo-hydroxybornane) (13) was isolated from A. vulgaris L. together with camphor, β-thujone and myrcene [33].

Sesquiterpene Lactones

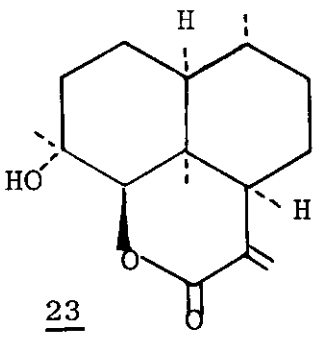
Another characteristic of Artemisia plants appears to be the presence of sesquiterpene lactones. α-Santonin (1) is present in various varieties of A.maritima L.like A.brevifolia [34] Wall, A.herba-alba Asso [35], A.kurramensis [36]. Various strains of A.kurramensis Qazilbash have been reported to yield both α and β-santonin (14) [27]. Artemisia maritima L., occurring in Kurram valley near Parachinar in Pakistan, is especially rich in santonin which occurs in the range of 1.27-2.75% [37]. Other sesquiterpene lactones, similar in structure to santonin, such as torrentin (15) [38] from A.herba-alba subspecies valentina [38] and artabin (16) [39,40] and arabsin (17) [41,42] from A.absinthium L. have been reported.

A.annua L. yielded sesquiterpene lactones, arteannuin B (18) [43], quinghaosu (19), an antimalarial [44,12] and other related compounds like quinghaosu I (20) [12], quinghaosu II (arteannuin B (18), [43,12], quinghaosu III (21) [12], quinghaosu IV (22) [12], quinghaosu V (23) [12] and quinghaosu acid (24) [12]. They all belong to amorphane (25) series which has a cis decalin skeleton with the isopropyl group trans to the hydrogen on the ring juncture [12].

Guaienolides like artabsin [45-47] (1-hydroxy-2,4(10)-dien-8,12-olide (26)), artemolin (27) [48] and diguaienolides like anabsin (28) [49,51], anabsinthin (29) [49,50], and absinthin (30) [46,47,52,53], have been isolated from A.absinthium L. Artabsin (26) and absinthin were also isolated from A.sieversiana Ehrh. ex Willd [54]. Artabsin (26) is a prochamazulenogen [55]. A guaienolide, seiversin was isolated from A.sieversiana Ehrh. ex Willd. [56] and its structure was shown to be 4-hydroxy-8-acetoxy-guaia-1(2), 9(10) dien-6,12-olide. Sesquiterpene ketone, psilostachyin C (31) was isolated [57] from Yugoslavian A.vulgaris L. which did not contain vulgarin (2). New sesquiterpene lactones, herbolides A, B, and C (32-34) were isolated [58] from A.herba-alba Asso. A term pelanolide [52] was suggested for a group of sesquiterpene lactones, isolated from A.absinthium L. and these materials were called ketopelenolide a ($C_{15}H_{22}O_3$; m.p. 114° $[\alpha]_D^{20}$ -227°, an oxolactone), and hydroxypelenolide a ($C_{15}H_{24}O_3$, m.p. 98°). A yellow lactone ($C_{30}H_{36}O_5$, m.p. 207°) and a keto lactone ($C_{15}H_{22}O_3$, m.p. 172°) were also isolated along with few other compounds from A.absinthium L. but the chemical structures of these compounds were not determined [59].

Three new garmacranolides 3β,8α -dihydroxy-6β H,7α H, 11β -H-germacran-4(14), 9(10)-dien-6,12-olide (35), the corresponding 3-oxo-olide (36) and 1β -hydroxy-6β H, 7α H, 11 H, germacan-4 (5), 10(5)-dien-6,12-olide (37) have been isolated from A.herba-alba Asso occuring in Sinai desert [60].

Artemolin (27) was also isolated from A.sieversiana Ehrh. ex Willd [61].



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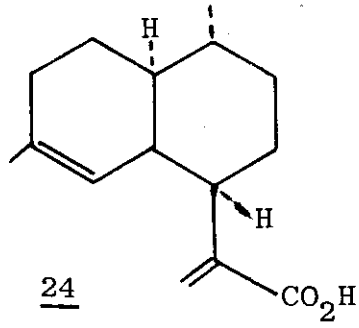

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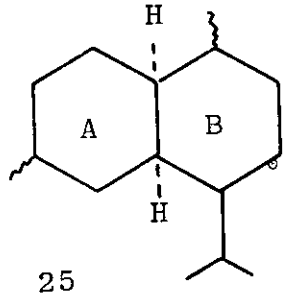

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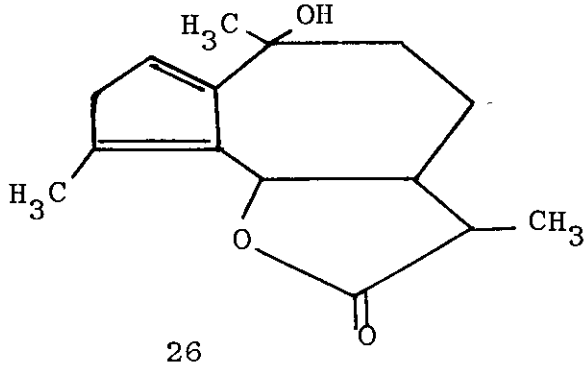

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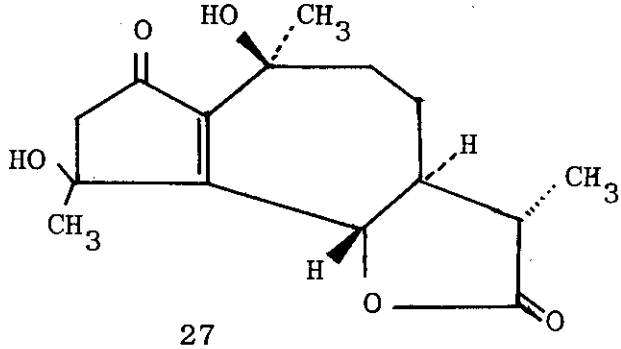

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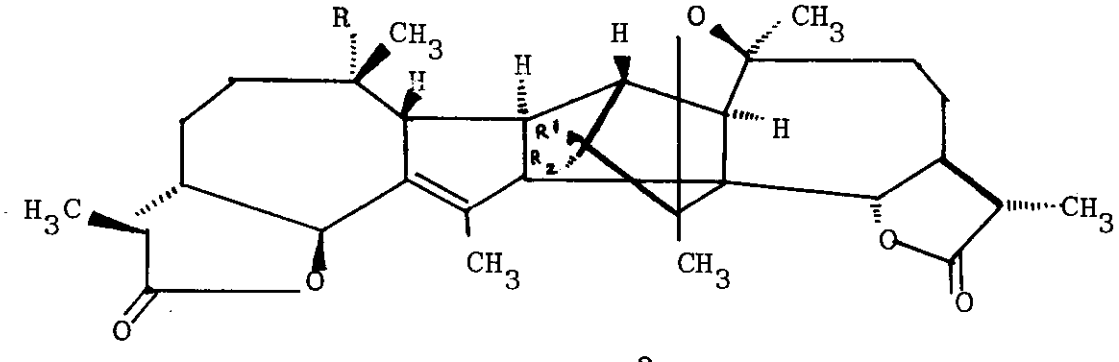
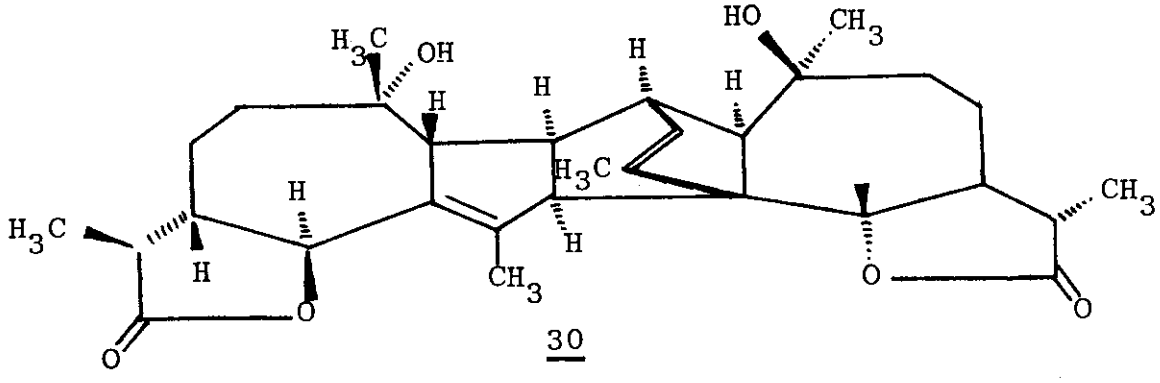


(28) R = R = OH, R^2 = H

(29) R = OH, R^1 = R^2 = H



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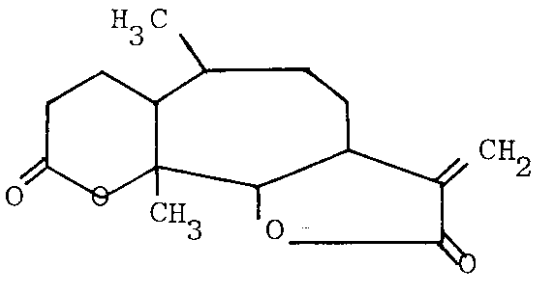

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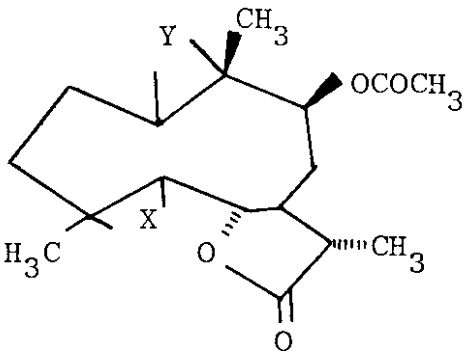


32 A X = Y = BOND

33 A X = BOND Y = O

34 A X O Y = BOND

Flavones

A new flavanol, quercetagetin-3',4' 6,7-tetramethyl ether (38) was isolated from A.annua [62]. A compound, artemetin, isolated from A.absinthium L. was identified as 5-hydroxy-3,3', 4',6,7-pentamethoxy-flavone [63]. 5,4'-Dihydroxy - 6,7,3'-trimethoxy flavone (39) was isolated from A.herba-alba Asso [64]. The flavonoids were extracted with hot water from A.scoparia Waldst. and Kit. and separated by preparative paper and polyamide column chromatography. Identification was done spectrometrically using ionizing and complex forming reagents. The leaves and anthodia (flower heads) contained more flavonoid components than did the stem. The major components of anthodia were rutin, quercetin-3-glucogalactoside, and a group of aglycons. Other identified flavonoids were kaempferol-3-glycogalactoside, quercetin-3, 7-rutinosoga-lactoside and quercetin-3,7-rutinosodigalactoside. The major component, independent of growth stage or plant organ, was quercetin-3-glucogalactoside, 0.61% of which occurred in leaves at the beginning of the growing season [65].

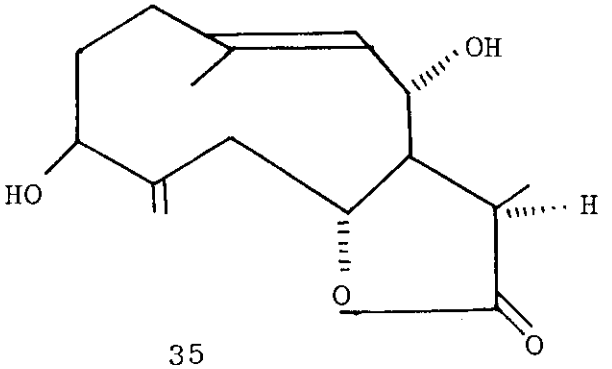
4.Coumarins

Various coumarins have been reported in Artemisia plants. Scoparone (6,7-dimethoxycoumarin) (40) occurs in A.scoparia Waldst. and Kit. [11, 66-69] but shows no anthelmintic activity [67]. Scoparone, present in A. capillaris Thunb [70-73], was also identified in A.japonica Thunb. (cf. A.parviflora Roxb. in Pakistan [2]) and in the Japanese drug, Inchinko, derived from this plant, alogwith capillarin (43) [73] and in A.parviflora Roxb. [74]. Scoparone was also identified recently [75] in A. elegantissima, occurring in Islamabad area of Pakistan.

Scopoletin (6-methoxy-7-hydroxy-coumarin) (41) was identified in A. parviflora Roxb. [74], A.japonica [76] and A.persica Boiss [77], while esculetin, 6,7-dihydroxy-coumarin (42) occurred in A.parviflora Roxb .[74].

5.Furans

A new sesquiterpenoid, 2-(2-methy -2-vinyl-tetrahydrofur-5-yl)-2,6,6-trimethyl-2,6-dihydro-pyr-3-one (44) was isolated from A.pallens Wall.



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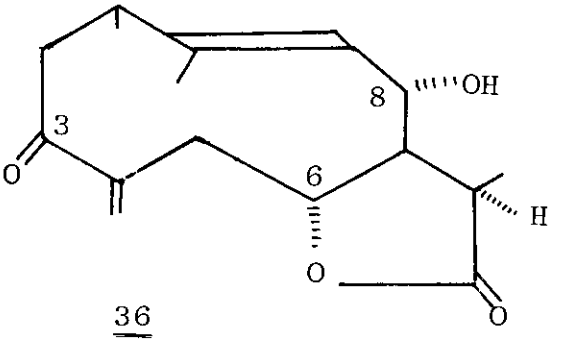

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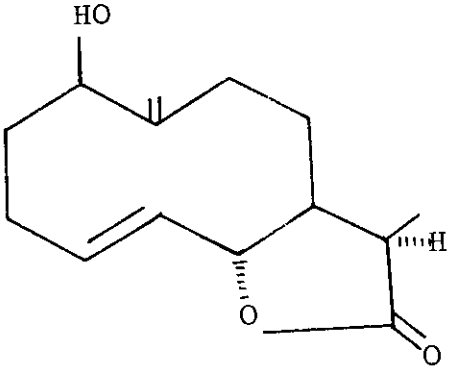

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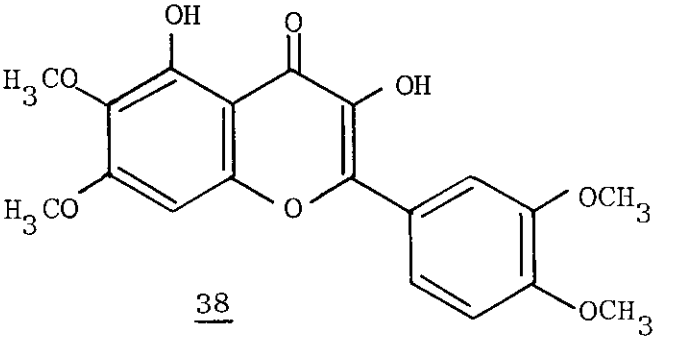

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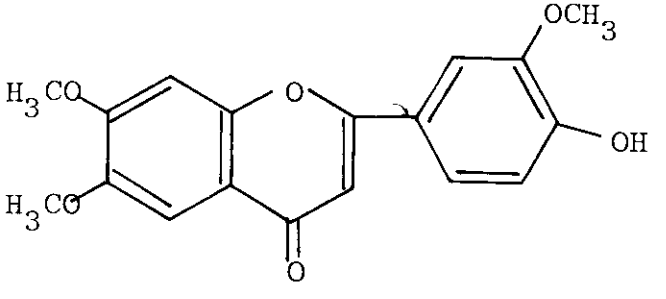

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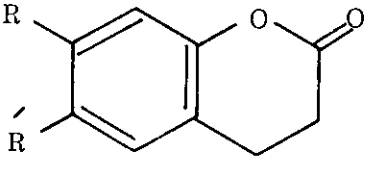
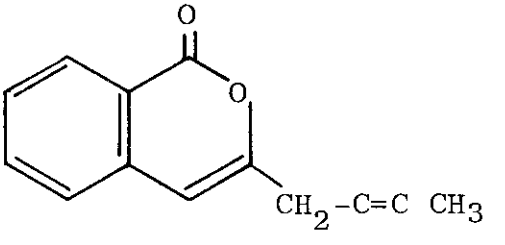


40 R = R = OCH_3

41 R = OCH_3, R = OH

42 R = R = OH



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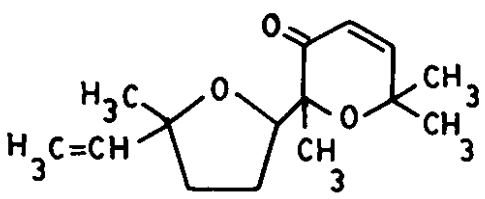

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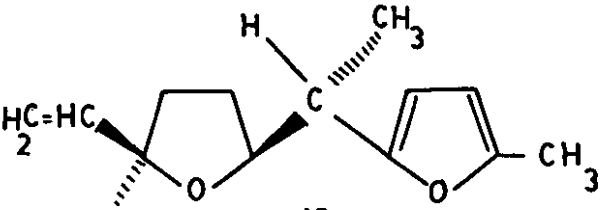

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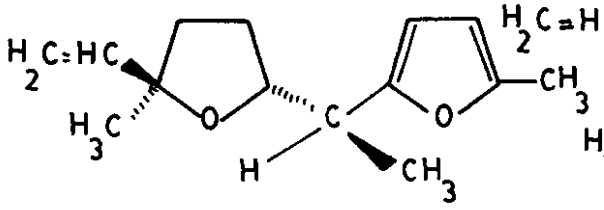

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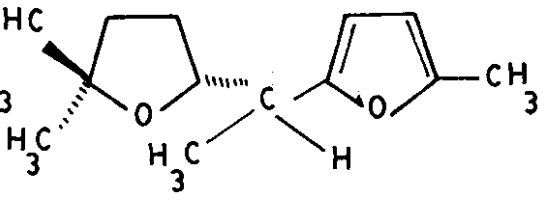

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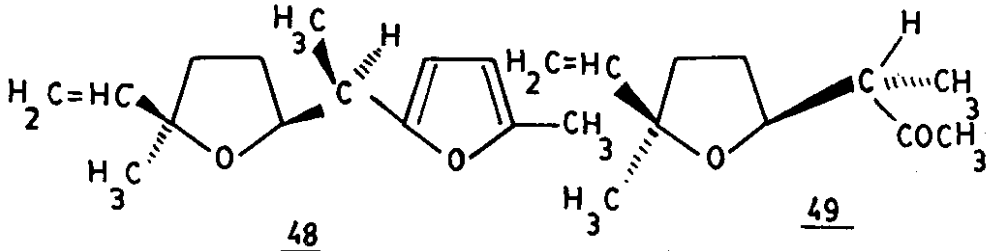

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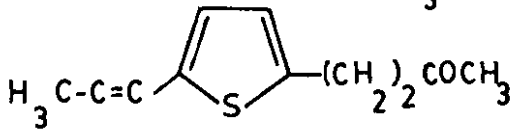

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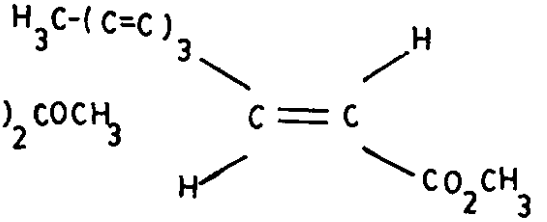

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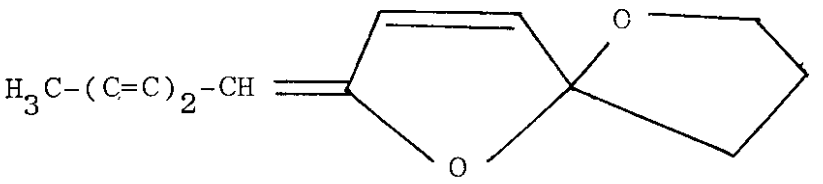

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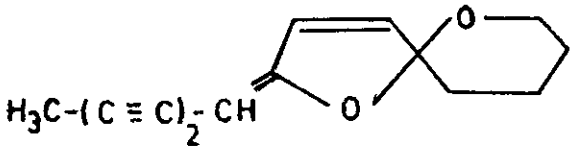

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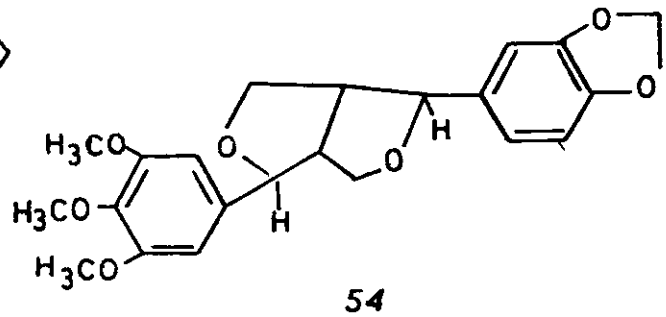

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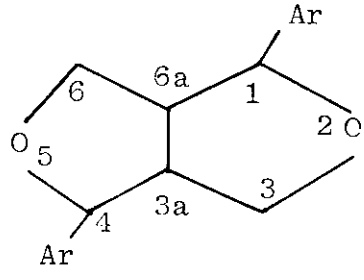

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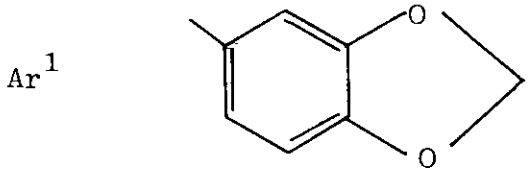
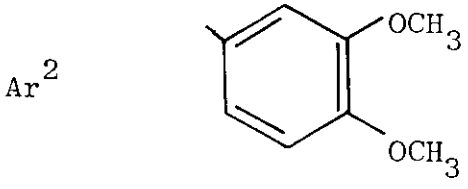
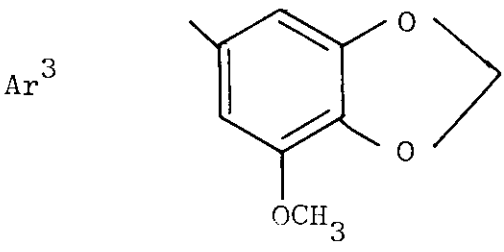
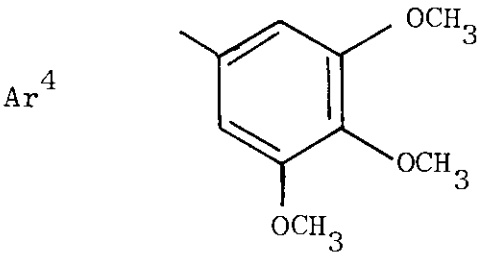








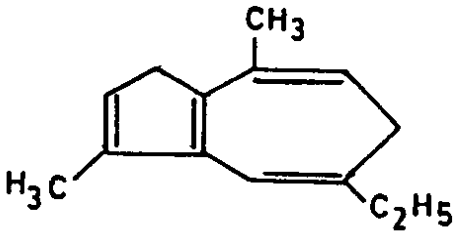
[78]. All four stereoisomers of davenafuran (45-48) were isolated from A.pallens Wall. and their structures and absolute configurations were established by synthesis from linalool oxides of known configurations [79]. A C_{11}-terpenoid, nordavanone (49), was also isolated from A.pallens Wall. and its structure was obtained by n.m.r. spectrometry and synthesis [80].

6.Acetylenes

The roots of A.absinthium L. contained acetylenic thiophene derivative (50) alongwith (51) and trans spiroketalenol ethers (52,53) [81]. Dehydromatricaria ester (Methyl n-decenetriyonate) was identified in the essential oil from the roots of A.vulgaris L. [82].

7.Miscellaneous Compounds

Thirteen tetrahydrofurfuran lignans were isolated from the roots of A. absinthium L. Four of these, stereoisomers of sesartemin (54) (1- (3,4-methylenedioxy-5-methoxy-phenyl-4-(3,4,5-trimethoxyphenyl)-tetrahydro-1H,3H-furo(3,4-c) furan) were new lignans while other compounds contained three other types of aromatic substituents on tetrahydro-1H, 3H-furo [3,4-c] furan (55) [83]. 3, 6-Dihydrochamazulene (56) and 5,6-



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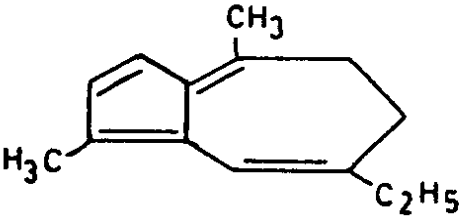

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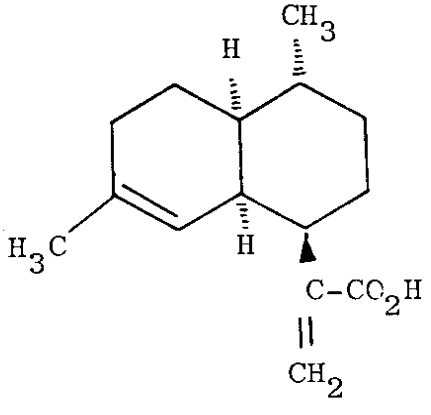

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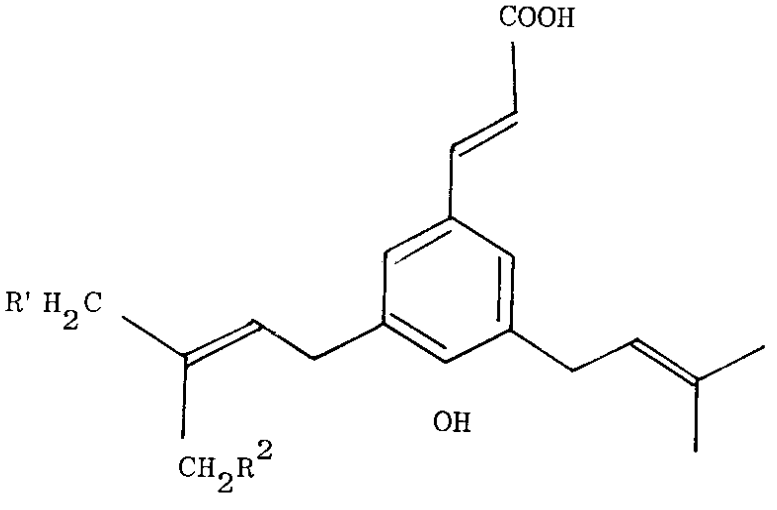

(59) R^1 = OH, R^2 = H

(60) R^1 = H, R^2 = OH

di-hydrochamazulene (57) were isolated from A.absinthium L. [84].

Stigmasterol and β-sitosteral were also reported [34] in A.herba alba Asso which was suggested to be important as fodder for sheep in the plateau regions of Algeria where it grows abundantly and is rich in aspartic and glutamic acids [85]. Artemisia sieversiana contains 15.5% protein and 5.12% fat and is a good material for silage [86]

Artemisia annua also yielded artemi-nic acid (58) [87]. Two new choleretic principles named cappilartemisin A (58) and (60) were isolated from Artemisia capillaris Herba and showed more potent and long lasting choleretic activity than sodium dehydracholate [88].

Economic Importance

Since use of santonin has decreased as an anthelmintic drug, therefore, it is important to find alternate uses of santonin or other synthetic compounds based on santonin, and also of other Artemisia plants in Pakistan. These plants could be used for isolation of essential oils as artemisia oils are used in perfumery

industry. Roches has created recently a fragrance for men which contains artemisia oil as one of the components [89]. Artemisia oil is already obtained as a by-product of santonin. The manufacture of the essential oils from other Artemisia plants for the perfumery industry or for export purposes should be examined.

For economic exploitation of these plants, however, it is necessary to survey various Artemisia species in northern areas of Pakistan and to determine their available quantities.

Artemisia plants are also of medicinal value and more research work is necessary to isolate and evaluate new drugs from these plants. A.herba-alba and A.sieversiana can also be used as fodder.

Acknowledgement

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