15), 1.45 (dd, $^2J = 15$ Hz, $^3J = 5.1$ Hz, 1 H, H_A-2), 1.48 (m, 1 H, H_A-8), 1.59 (m,br, 1 H, H_B-14), 1.60 (m, br, 1 H, H_B-14), 1.62 (dd, $^3J = 7.0$ Hz, 2.5 Hz, 1 H, H-16), 1.78 (m, br, 1 H, H_B-8), 2.05 (dd, $^2J = 17.0$ Hz, $^3J = 3.5$ Hz, 1 H, H_A-4), 2.06 (dd, $^2J = 15$ Hz, $^3J = 2.0$ Hz, H_B-2), 2.08 (m, br, 1 H, H-13), 2.17 (d, $^2J = 17.0$ Hz, 1 H, H_A-18), 2.18 (d, $^2J = 17.0$ Hz, 1 H, H_B-18), 2.34 (dd, $^2J = 17$ Hz, $^3J = 5.5$ Hz, 1 H, H_B-4), 2.43 (m, br, 1 H, H_A-3), 2.48 (m, br, 1 H, H_B-3), 2.64 (dd, $^3J = 1.5$ Hz, 2.0 Hz, 1 H, H-10), 2.78 (dd, $^3J = 12.0$ Hz, 6.1 Hz, 1 H, H-9), 3.1 (m, br, 1 H, H-3), 4.05 (q,dd, $^3J = 6.5$ Hz, 5.5 Hz, 4 Hz, 1 H, H-22), 4.18 (s, 1 H, 19-OH), 4.52 (d, $^3J = 6.5$ Hz, 1 H, 22-OH), 4.58 (d, $^3J = 2.8$ Hz, 1 H, 3-OH), 5.38 (d, $^3J = 0.5$ Hz, 1 H, H-12), 5.51 (dd, $^3J = 6.1$ Hz, 1.0 Hz, 1 H, H-5), 5.53 (ddd, $^3J = 16.5$ Hz, 5.5 Hz, $^4J = 1.8$ Hz, 1 H, H-21), 5.64 (dd, $^3J = 16.5$ Hz, $^4J = 2.5$ Hz, 1 H, H-20), ^{13}C-NMR (DMSO-d_6, δ, ppm): 14.52 (C-25), 22.3 (C-14), 22.5 (C-15), 24.20 (C-23), 27.36 (C-7), 27.70 (C-8), 29.10 (C-24), 36.17 (C-4), 39.0 (C-18), 41.56 (C-2), 46.11 (C-13), 48.30 (C-6), 53.45 (C-9), 55.5 (C-13), 60.12 (C-16), 63.31 (C-3), 66.50 (C-22), 73.46 (C-19), 122.2 (C-10), 124.73 (C-12), 131.21 (C-21), 136.20 (C-20), 145.90 (C-5), 157.30 (C-11), 204.29 (C-1). $[\alpha]_D^{25}$ (MeOH, 2.993 mg/ml): +169.1°. CD (Δε, nm): +9.2 (245), −51.1 (310).

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Alkaloids and bioactivity of *Papaver triniifolium*

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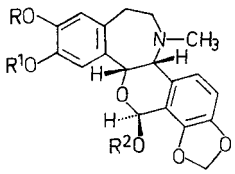
Our previous studies on the endemic species of the section Miltantha, *Papaver triniifolium* Boiss. (Papaveraceae) growing in Turkey revealed the existence of several chemotypes which yielded rhoeadine, morphinane, aporphine and proaporphine types as major alkaloids [1]. Recently the existence of a chemotype containing medicinally important (−)-α-narcotine and papaverine as major alkaloids has been shown [2]. In this work, we report the isolation and characterization of the alkaloids of another sample of *Papaver triniifolium* collected from Beypazarı in Ankara.

Table: Brine shrimp bioassay results of the tertiary and quaternary alkaloidal extracts and compounds 1, 2, 7, 8 of the aerial parts of *P. triniifolium*

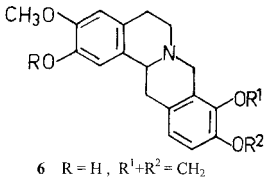
Type of Extracts	ppm	LC_{50}
Tertiary alkaloidal extract	1000 : 100 : 10	515.02
Quaternary alkaloidal extract	1000 : 100 : 10	518.24
1	250 : 25 : 2.5	297.04
2	250 : 25 : 2.5	236.91
7	250 : 25 : 2.5	>1000
8	250 : 25 : 2.5	375.28
Berberine chloride*	250 : 25 : 2.5	8.63

* positive control

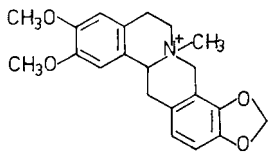
The major alkaloids of the aerial parts of this sample have been shown to be rhoeadine [(+)-oreogenine (**1**), (+)-rhoeagenine (**2**)] type. Other alkaloids are three rhoeadines [(+)-oreodine (**3**), (+)-O-ethyloreogenine (**4**), (+)-O-ethyl-rhoeagenine (**5**)], four protoberberines [(−)-cheilanthifoline (**6**), (−)-sinactine (**7**), (−)-*N*-methylsinactine (**8**), (−)-isocorypalmine (**9**)], one phthalideisoquinoline [(−)-α-narcotine (**10**)] and one benzylisoquinoline [crykonisine (**11**)]. This is the first report of the isolation of **1**, **2**, **8**, **9**, **11** from *P. triniifolium* and **8**, **9**, **11** from the section Miltantha. The presence of **8** and **11** has been shown in the Papaveraceae family for the first time. Previously, compound **8** has been isolated from *Fumaria officinalis* (Fumariaceae), whereas compound **11** has been obtained from



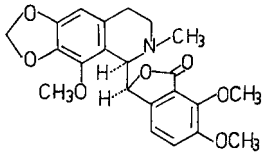
1 R = R^1 = CH$_3$, R^2 = H
2 R+R^1 = CH$_2$, R^2 = H
3 R = R^1 = R^2 = CH$_3$
4 R = R^1 = CH$_3$, R^2 = C$_2$H$_5$
5 R+R^1 = CH$_2$, R^2 = C$_2$H$_5$



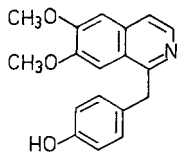
6 R = H, R^1+R^2 = CH$_2$
7 R = CH$_3$, R^1+R^2 = CH$_2$
9 R = H, R^1 = R^2 = CH$_3$



8



10



11

Cryptocarya konishii (Lauraceae) [3–4]. Compounds **4**, **5** have been considered as artifacts formed during the isolation procedure. Tertiary and quaternary alkaloidal extracts of the aerial parts of *P. triniifolium* displayed toxicity in the brine shrimp bioassay.

Experimental

1. Plant material

P. triniifolium Boiss. was collected, in the flowering stage, at Beypazarı in Ankara (Inner Anatolia) in June 1995. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy of Istanbul University (ISTE 68174).

2. Extraction, isolation and identification

The dried and powdered aerial parts (1.26 kg) were percolated with ethanol. Tertiary and quaternary alkaloidal extracts were then obtained in the usual manner as 13.11 g and 0.520 g, respectively [2]. Separation of **1** (940 mg), **2** (570 mg), **3** (29 mg), **4** (34 mg), **5** (5.4 mg), **6** (105 mg), **7** (160 mg), **9** (14 mg), **10** (110 mg), **11** (3.9 mg) was achieved by CC on silica gel (200 g) eluting with $CHCl_3$ and $CHCl_3$: MeOH (9 : 1). 59 fractions of 50 ml were collected. Preparative TLC was then used for further separation and purification on silica gel with systems C_6H_6 : Me_2CO : MeOH (8 : 1 : 1), C_6H_6 : MeOH : NH_3 (95 : 5 : 0.5), cyclohexane : $CHCl_3$: $(C_2H_5)_2NH$ (7 : 2 : 1), $CHCl_3$: MeOH (95 : 5). Separation of quaternary alkaloid **8** (18 mg) was by preparative TLC on silica gel using the system $CHCl_3$: MeOH (8 : 2) . The structures of the alkaloids were confirmed by spectral (UV, IR, ^{1}H NMR, and EIMS) and physical (α_D^{20}, m.p.) methods [3–7]. Spectral methods as well as TLC comparison were used for the identification of the alkaloids. The ^{1}H NMR data of **4** and IR, EIMS data of **11** are presented here for the first time

Compound **4**. ^{1}H NMR (200 MHz, DMSO): $\delta = 1.17$ (3 H, t, J = 7 Hz, OEt), 2.21 (3 H, s, NMe), 3.69 (1 H, d, $J_{1,2} = 2$ Hz, H-2), 5.00 (1 H, d, $J_{1,2} = 2$ Hz, H-1), 5.75 (1 H, s, H-14), 5.97 (2 H, s, A-ring OCH_2O), 5.99 and 6.11 (2 H, dd, J = 1.2 Hz, D-ring OCH_2O), 6.75 (1 H, s, H-6), 6.81 (1 H, s, H-9), 6.84 (2H, ABq, H-10,11).

Compound **11**: IR ($CHCl_3$, cm^{-1}): ν = 3375, 2920, 2363, 1610, 1511, 1479, 1421, 1272, 1159, 1085, 1040, 881, 850. EIMS m/z (rel. int.): 295 (87), 294 (100), 280 (75), 264 (21), 249 (20), 236 (27), 220 (23), 190 (24), 178 (13), 148 (16), 107 (12).

3. Cytotoxicity assay

Tertiary and quaternary alkaloidal extracts of *P. triniifolium* were screened for their cytotoxicity using *Artemia salina* (brine shrimp) [8]. LC_{50} values of these extracts and compounds **1**, **2**, **7**, **8** are shown in the Table. The control used for comparison was berberine chloride which has a strong cytotoxic activity. Both tertiary and quaternary alkaloidal extracts showed significant lethality to brine shrimp larvae. Compounds **1** and **2** were both found out to be responsible for the cytotoxic activity of the tertiary alkaloid extract and compound **8** was found out to be responsible for the cytotoxic activity of the quaternary alkaloid extract. Though compound **7** has been shown to be inactive, its quaternary derivative, compound **8**, was proven to be active.

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