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THE ALKALOIDAL CONSTITUENTS OF *PAPAVER BRACTEATUM* ARYA II

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Key Word Index—*Papaver bracteatum*; Papaveraceae; alkaloids; *O*-methylflavinantine; thebaine; 14β-hydroxycodeine; codeine; neopine; alpinigenine; protopine.

Abstract—Seven alkaloids were isolated from *Papaver bracteatum* Arya II, six of which: thebaine, 14β-hydroxycodeine, codeine, neopine, alpinigenine and protopine, have been previously found to be present in other types of this species. It is the first report of the isolation of *O*-methylflavinantine from *P. bracteatum*.

INTRODUCTION

Papaver bracteatum alkaloids have been extensively investigated [1]. Two main sources of seeds were available, which were identified as Arya I and Arya II [2], both containing thebaine in different concentrations and the chemical variability in thebaine content has been reported for both types [3, 4]. The chemical constituents of Arya I have been reported earlier [5] and in our hands, a population which differs from both Arya I and II and originating from the Polour region (north of Tehran) [6] was found to contain about 1% of thebaine and as high as 0.2% of alpinigenine (**6**); these results are reported elsewhere [7].

Under the initiative of the Special Action Office for Drug Abuse Prevention (Office of the President of the United States), a pilot large scale extraction of some 3.1 tons of capsules of *Papaver bracteatum* Lindl. Arya II collected in the Mahabad region (western Iran) [6], was carried out in Israel in the summer of 1975, in collaboration with a local company, Plantex Ltd. Natanya. The aim of this large scale pilot experiment was to show the commercial feasibility of the thebaine extraction process from the plant bracteatum which was investigated as an agricultural crop and a new source for thebaine (**1**). The thebaine content of this batch of Arya II capsules was found to be 2.3%, and the average moisture content 9%.

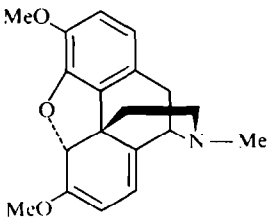
From this pilot experiment, large quantities of mother liquors left from the separation and crystallization of the thebaine were available. This paper deals with the chemical analysis of these mother liquors and reports the chemical constituents other than thebaine present in Arya II.

RESULTS

The toluene concentrate of the mother liquors was evaporated to dryness, and the alkaloidal fraction was separated and resolved by repeated chromatography on alumina columns. The compounds which were isolated and fully characterized are: additional quantities of thebaine (**1**), which was still the main component of the mixtures; 14β-hydroxycodeine (**4**); codeine (**2**); neopine (**7**); alpinigenine (**6**); protopine (**9**); and *O*-methylflavinantine (**10**).

Among the different constituents isolated and identified so far, 14β-hydroxycodeine was predominant after thebaine, it was identified by comparison with an authentic sample obtained from the reduction of 14β-hydroxycodeinone prepared from thebaine. Codeine and neopine, identified by comparison with authentic samples, were present in smaller quantities, whereas alpinigenine and protopine were also identified using authentic samples and occurred in trace amounts. *O*-Methylflavinantine was identified by detailed spectroscopic analysis and was also found in a very small quantity.

Concerning *O*-methylflavinantine (**10**), it is the first instance that this compound has been isolated from *P. bracteatum*. The high resolution mass spectrometry afforded the $C_{20}H_{23}NO_4$ molecular ion and the appropriate fragmentation. Its ^{1}H NMR spectrum carried out at 270 MHz showed the presence of four low field one proton singlets each, at δ 6.82, 6.64, 6.36 and 6.35 related to the aromatic ring and the cross-conjugated cyclohexadienone protons, three singlets at δ 3.89, 3.86 and 3.81 for the three methoxy groups, and one N-Me singlet at δ 2.51. The assignments of all other signals were confirmed by selective decoupling of each proton signal in turn. The C-9 proton appeared as a doublet at δ 3.77 (J = 6 Hz) collapsing to a singlet upon irradiation at δ 3.08 which is the location related to the 10α-H (a quartet). The latter turns to a doublet (J = 18 Hz for gem. coupling) upon reciprocal irradiation. A signal at δ 3.39 (d, J = 18 Hz) is related to the 10β-H which collapses to a singlet upon irradiation at δ 3.08, no observable coupling taking place with 9-H. A two proton signal at δ 2.64 was assigned to the two 16-H. Narrowing of this signal took place upon irradiation at δ 1.91 (a complex multiplet assigned to the two 15-H). Inverse irradiation gave a double doublet at δ 1.85 and 1.96 (J = 12.5 Hz gem. coupling). The UV spectrum was λ_{max} 236 and 282 nm (ε 12800 and 5800) the former being related to the α-methoxy cross-conjugated chromophore of **10**. The IR supported this structure: $\nu_{max}^{CHCl_3}$ 1668, 1649 and 1623 cm^{-1}. Such a compound has also been isolated from a quite different plant and given the name of sebiferine [8], and some of its pharmacology has been recently described [9].



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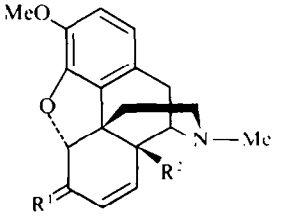
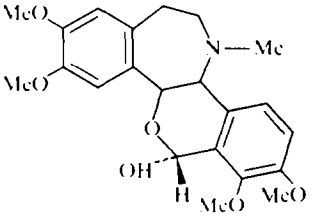


2 R^1 = βH, αOH, R^2 = H
3 R^1 = O, R^2 = H
4 R^1 = βH, αOH, R^2 = OH
5 R^1 = O, R^2 = OH



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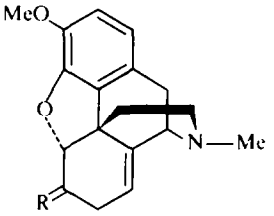
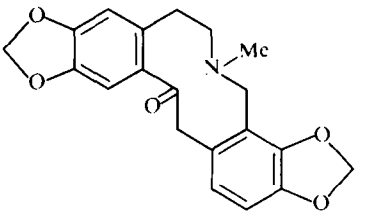


7 R = βH, αOH
8 R = O



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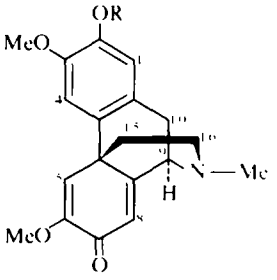
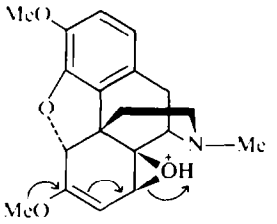


10 R = Me
11 R = H



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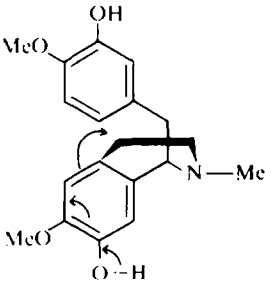

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DISCUSSION

The availability of large quantities of mother liquors left from the thebaine isolation of the large scale processing of the Arya II capsules enabled the detection and chemical identification of compounds present in very small quantities. Knowing their identity would facilitate their subsequent detection in the capsules of the various populations of plants and enable tests to be performed on small samples. An easier chemical identification procedure of the different chemotypes occurring in the species might ensue.

The chemical constituents of Arya I [5] and Arya II were found to be fairly similar. The outstanding feature is the presence of codeine and neopine, two products of the demethylation of thebaine; however, no morphine has been detected in any of those two populations. It can be assumed that the demethylating enzyme present in *P. bracteatum* is extremely sluggish in distinction to *P. somniferum*, in which the demethylation process is predominant. This sluggishness accounts for the overwhelming formation of the baine in the former plant in up to 98% of the alkaloidal content.

The occurrence of 14β-hydroxycodeine (**4**) in the two populations, Arya I and Arya II, indicates a new direction in the biosynthetic sequence as referred by the group of Salemink [5]. They have suggested neopinone (**8**) as a possible intermediate towards the formation of the 14β-hydroxycodeinone (**5**). In such a case a photosensitized oxygenation involving singlet oxygen might be considered, leading to the formation of the product **5** [10, 11]. An alternative possibility would be through the formation of an oxygenated species as shown in **12**, which should then undergo a demethylation process to form the 14β-hydroxycodeinone as indicated by the arrows, followed by the usual reduction to the codeine analogue. Such oxidative transformations have been suggested and discussed with different type of natural compounds [12, 13].

The formation of *O*-methylflavinantine (**10**) can be accounted for by an oxidative coupling of (−)reticuline (**13**) which is different from the one that leads to salutaridine and thebaine. Whereas in the latter case *ortho-para* coupling takes place, in the former, a *para-para* oxidative coupling has to occur producing pallidine (**11**) (an isomer of salutaridine) which through methylation should produce the *O*-methylflavinantine (**10**).

When comparing the occurrence of the above compounds to those previously identified in Arya I [5], it was found that protopine and *O*-methylflavinantine were found in Arya II, whereas no 14β-hydroxycodeinone was

detected. Since codeinone is known to be unstable, specially when in solution, the detection of the 14β-hydroxycodeinone reported in Arya I [5] could not have been repeated in our case. In distinction to Arya II in which alpinigenine was found to occur in minute quantities, Arya I is richer in this compound (0.12%) [5], whereas the type collected in the Polour region contained alpinigenine in concentrations as high as 0.2%.

EXPERIMENTAL

Mps were taken on a Fisher Johns apparatus. Chromatography was performed on neutral alumina Woelm activity III unless otherwise stated. TLC were carried on chromatoplates of silica gel PF_{254} (Merck) developed in toluene–acetone–EtOH–NH_4OH (20:30:15:1). NMR spectra were determined on Bruker HFX-10 90 MHz and WH-270 instruments for $CDCl_3$ solns containing TMS as internal standard. MS were done on an improved Atlas CH4 instrument for low resolution and a Varian MAT 731 High Resolution Mass Spectrometer under the direction of Dr. Z. Zaretskii.

Isolation procedure. 4.5 l. of the toluene concentrate of the mother liquors from the thebaine isolation were evapd to dryness and the residue dissolved in MeOH–H_2O (2.5 l.) and acidified to pH 2 (aq. HCl). The solution was extracted with hexane and then with ether. The aqueous layer was made basic with a solution of ammonia and then extracted with $CHCl_3$. The $CHCl_3$ layer was dried and evaporated to give 137 g of residue. This residue was chromatographed on a column of alumina (3 kg Alcoa) and was eluted with the following solvent mixtures: C_6H_6, C_6H_6–$CHCl_3$ (1:1), $CHCl_3$, 1% MeOH $CHCl_3$ and 10% MeOH $CHCl_3$. From the first two, only traces of material were obtained. The initial few fractions from $CHCl_3$ afforded mainly additional thebaine (8.5 g). The following fractions afforded complex mixtures (48.5 g) which were further chromatographed for careful separation on alumina. Elution of this column was done with solvent mixtures of Et_2O–CH_2Cl_2 while gradually increasing the concentration of the latter. The content of the fractions was followed by chromatoplates.

Fraction 11 consisted again mainly of thebaine which was separated by crystallization (EtOH). The residue obtained from the mother liquor of this crystallization (0.5 g) was further chromatographed on the same support using Et_2O. A pure product was obtained, after crystallization in CH_2Cl_2–petrol, which was identified as alpinigenine. Mp 187°, $[\alpha]_D$ + 226° (*c*, 1.0), comparison with an authentic sample showed same R_f on TLC and identical NMR spectra. The mother liquor from the separation of alpinigenine (275 mg) was chromatographed again on the same support. Elution with CH_2Cl_2 gave few fractions which after crystallization from MeOH–CH_2Cl_2 gave a small amount of crystalline compound (32 mg), mp 205–207° which was shown following spectroscopic measurements to be identical with protopine (**9**) by direct comparison with an authentic sample.

Fractions 12–24 of the previous chromatography (17 g) consist again mainly of thebaine. The following fractions 25–28 were mixtures (2.2 g) whereas the fractions 29–35 (4.6 g) were combined for further separation. Part of this quantity (100 mg) was applied on thick layer chromatoplates, and developed with the same solvent mixture given above: 52 mg of a pure compound was obtained, mp 153–156° (crystal. from acetone–hexane). The compound was identified as 14β-hydroxycodeine by direct comparison with a sample obtained by reduction of 14β-hydroxycodeinone with $NaBH_4$ in MeOH: mp 157°, and no depression upon mixture with the natural compound; identical NMR spectra; and MS: M^+ 315 as required. A second component was isolated from the same chromatoplates (16 mg) which was identified as codeine by comparison with an authentic sample.

Fractions 46–47 (70 mg) were also purified on thick layer chromatoplates and consisted mainly of neopine: mp 126–127°, R_f on TLC, and NMR were found identical with an authentic sample. Having completed the identification of these five compounds, it can be stated that fractions 25–28 (2.2 g) consisted as indicated by TLC of mixtures of 14β-hydroxycodeine, codeine and neopine.

Fractions 56–57 (1.3 g) were purified again on alumina. Elution with a mixture of Et_2O–CH_2Cl_2 (1:1) gave a fraction (0.65 g) which was further separated on the same support. Elution with a mixture of Et_2O–CH_2Cl_2 (8:3) gave a small fraction (76 mg) which was purified on preparative plates, using the same silica and procedure, to give a compound having the empirical formula $C_{20}H_{23}NO_4$ found by high resolution mass spectrometry: MW 341.164. This compound, one spot on chromatoplate, failed to crystallize. It was identified as *O*-methylflavinantine by NMR, UV, IR and MS fragmentation [14]. MS: M$^+$ 341.164 (57%), *m/e* 326.138 (18) ($M - CH_3$) 313.167 (13) ($M^+ - CO$); 298.145 (27) ($M^+ - CH_3 - CO$); 284.108 (3) ($M^+ - C_3H_7N$); 282.149 (15) ($M^+ - CO - OCH_3$); 58.066 (12) (C_3H_8N); 57.059 (2) (C_3H_7N).

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