Table II—Theoretical, Experimental, and Computer-Calculated Values of D_m for Benzene–Acetone and Benzene–Methyl Isobutyl Ketone Binary Systems

	Benzene–Acetone D_m				Benzene–Methyl Isobutyl Ketone D_m		
X_2	Theoretical	Experimental	Computer Calculated[a]	X_2	Theoretical	Experimental	Computer Calculated
0.000	2.30	2.30	2.19	0.000	2.30	2.30	2.27
0.284	3.39	5.83	5.85	0.190	3.22	4.28	4.23
0.549	5.24	10.42	10.20	0.420	4.72	6.66	6.63
0.784	9.37	15.65	15.14	0.690	7.33	9.59	9.50
0.850	11.02	—	—	0.800	8.88	—	—
0.900	13.18	—	—	0.900	10.62	—	—
0.950	16.19	—	—	0.950	11.67	—	—
1.000	20.90	20.90	20.98	1.000	12.89	12.90	12.86

[a] Based on computer-determined values of the coefficients. (See Ref. 2.)

system are plotted), because the ideal conditions under which the relationship was derived were not satisfied in real mixtures.

For example, the molar volumes of the two solvents change upon mixing. The electrical forces of the surrounding molecules exert an appreciable effect on each other. Consequently, the molar polarizations of the solvents also change upon mixing. Hence, the D_m values calculated from theoretical values of a, b, c, and d are significantly different from the experimentally determined D_m values. Therefore, it becomes necessary to measure the D_m values experimentally and subject them, along with the X_1 and X_2 values, to computer analysis to determine the values of the coefficients that will produce the best fit of the relationship to the experimental data.

The best values of the a, b, c, and d coefficients were determined by computer analysis (2) based on a series of laboratory experiments. The dielectric constant for any mixture of the benzene–acetone or the benzene–methyl isobutyl ketone binary solvent system can thus be predicted with the use of these values and Eq. 2. As reported in Table II, the predicted values (computer calculated) for D_m compare favorably with the experimentally determined values. This finding supports the usefulness of Eq. 2 for predicting D_m values of the binary solvent systems of the type studied here.

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Alkaloids of *Papaver orientale* and *Papaver pseudo-orientale*

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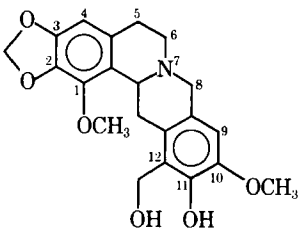
Abstract □ Dry latex of *Papaver orientale* was shown to contain 20% oripavine and 9% thebaine. Dry latex of *Papaver pseudo-orientale* contained two major alkaloids, isothebaine (11.7%) and orientalidine (0.5%), and the minor alkaloids bracteolin, salutaridine, Or_1, Or_2, PO-4, alborine (PO-5), and a novel alkaloid aryapavine.

Keyphrases □ *Papaver* alkaloids—isolation and identification from *P. orientale* and *P. pseudo-orientale* □ Aryapavine—isolation, identification from *Papaver pseudo-orientale*

As part of a broad study of the *Papaver* genus in Iran (1–3), the chemotaxonomy of *Papaver bracteatum*[1], *Papaver orientale*[1], and *Papaver pseudo-orientale*[1] has been studied.



I

Contradictory results exist in the literature with regard to the alkaloids of *P. bracteatum* and *P. orientale*. In addition to thebaine, other alkaloids have been reported in *P. bracteatum* (4–7). However, the present authors and others have demonstrated the existence of only thebaine (1, 3, 8) or a small amount of alpinigenine in addition to thebaine (2, 9–11) in *P. bracteatum*. Similar contradictory results have been reported for *P. orientale*. In addition to isothebaine,

[1] The plants were identified by P. Goldblat, Missouri Botanical Garden, St. Louis, Mo. Herbarium samples have been deposited in the Missouri Botanical Garden.

thebaine has been reported to exist in *P. orientale* (12). Some workers found oripavine in *P. orientale* (13, 14), while others reported the same alkaloid in *P. bracteatum* (4). These results suggest that the previous alkaloid accounts are unreliable with respect to species determination.

With the objective of clarifying present uncertainties, the authors undertook the study of the alkaloids of these species, keeping in mind the taxonomic studies of these plants.

Three species of section oxytona were classified according to their haploid chromosome number: *P. bracteatum*, $n = 7$; *P. orientale*, $n = 14$; and *P. pseudo-orientale*, $n = 21$[2].

In the present work, the alkaloid content of the dry latex of *P. orientale* and *P. pseudo-orientale* is reported. These wild plants grow in the mountains in north and northwest Iran.

EXPERIMENTAL[3]

Extraction of Alkaloids from *P. orientale*.—Dried latex (2 g), obtained by incision of the capsules of the plant growing in Takab (northwest Iran), was treated with dilute ammonia to obtain a soft paste and extracted by trituration with chloroform (3 × 20 ml). The extract was filtered and evaporated under reduced pressure. The residue (0.64 g) was chromatographed (TLC, silica gel), using ethyl acetate–methanol–ammonia (85:10:5) as the eluting solvent. The fractions were worked up and crystallized from appropriate solvents.

Extraction of Alkaloids from *P. pseudo-orientale*.—Dried latex (100 g), naturally excreted (as flakes) from capsules during maturation, was worked up as described for *P. orientale*. The residue obtained from the evaporation of chloroform was subjected to column chromatography on silica gel using chloroform–petroleum ether (50:50) as the eluting solvent at first and then gradually increasing the chloroform proportion. Finally, a mixture of chloroform–methanol (95:5) was used, and the proportion of methanol was gradually increased up to 50%. The fractions were further purified by TLC [ether–acetone–diethylamine (85:8:7)] and crystallized.

RESULTS AND DISCUSSION

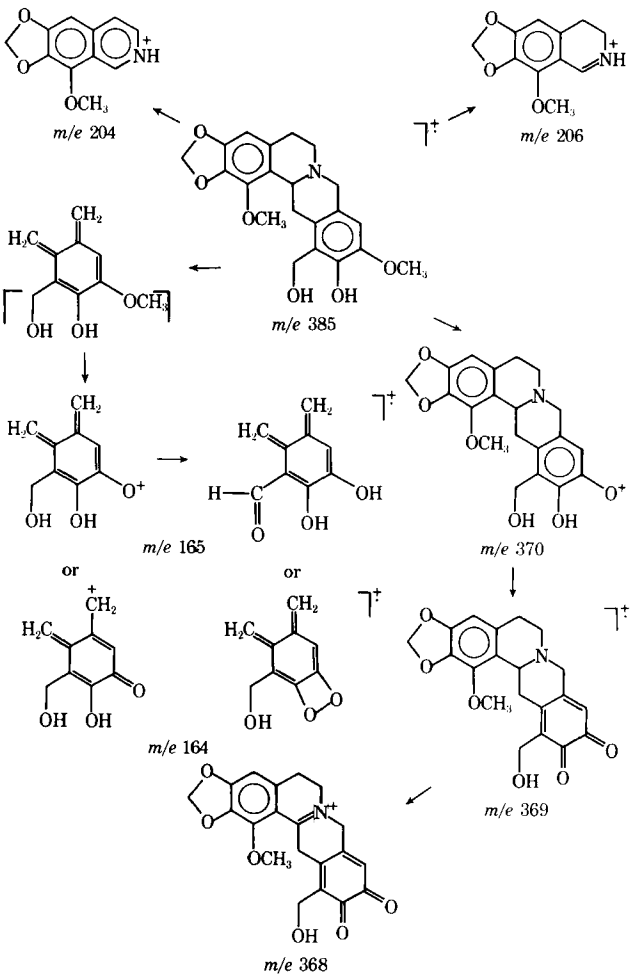
Alkaloids of *P. orientale*.—Two alkaloids were isolated and crystallized:

1. Oripavine (0.45 g, 20%), mp 201–203° (ethanol) [lit. (14) mp 200–201°]. The UV, IR, and NMR spectral properties of the separated alkaloid were identical with those of the authentic sample.
2. Thebaine (0.18 g, 9%), mp 193° (ethanol) [lit. (1–3) mp 193°].

Other samples of the plants collected from different regions of northwest Iran also had oripavine and thebaine; however, in one case, plants collected from Khalkhal (northwest Iran) had trace amounts of isothebaine in addition to oripavine and thebaine.

Alkaloids of *P. pseudo-orientale*.—The following alkaloids were characterized from the extensive studies of the dried latex:

1. Orientalidine, crystallized from ether (0.5 g, 0.5%), mp 192–195° [lit. (15, 16) mp 192–195°].
2. Isothebaine, crystallized from ethanol (11.7 g, 11.7%), mp 203–205° [lit. (15) mp 203–204°].
3. Bracteolin, crystallized from ether, mp 228–230° [lit. (15) mp 226–229°].
4. Salutaridine, crystallized from ether, mp 197–199° [lit. (15) mp 196–198°].
5. Or_1, crystallized from petroleum ether, mp 106–107° [lit. (15) mp 109°].



Scheme I

6. Or_2, crystallized from petroleum ether, mp 120–121° [lit. (15) mp 124–127°]; UV λ_{max} (ethanol): 285 (log ϵ 3.27) and 226 (log ϵ 3.12) nm; NMR ($CDCl_3$): δ 6.63 (s, 1H, aromatic), 3.98 (s, 3H, OCH_3), 3.43 (s, 3H, OCH_3), and 2.47 (s, 3H, NCH_3) ppm. The mass spectrum had a molecular ion at 333.
7. PO-4 (16), crystallized from acetone–ether, mp 300° [lit. (17) mp 300°].
8. Alborine (PO-5) (16), crystallized from methanol–ether, mp 300° [lit. (17) mp 300°].

The spectral data (UV, IR, NMR, and mass) of these alkaloids were identical with those reported in the literature (1–3, 15–17).

In addition to these alkaloids, a new alkaloid was also isolated, 11-demethylmecambridine (aryapavine) (I), mp 156–157° (acetone); UV λ_{max} (ethanol): 287 nm (log ϵ 3.87); NMR ($CDCl_3$): δ 6.62 (s, 1H, H_4), 6.43 (s, 1H, H_9), 5.97 (s, 2H, OCH_2O), 4.80 (s, 2H, CH_2O), 4.07 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), and 3.90–2.20 (m, 9H, aliphatic) ppm; IR (KBr): 3400 cm^{-1} (broad, hydrogen bonded hydroxy). The mass spectrum had peaks at *m/e* (%) 385 (M, 70), 370 (77), 369 (85), 368 (63), 206 (100), 204 (77), 165 (55), and 164 (77). Its empirical formula was $C_{21}H_{23}NO_6$. These data were in agreement with Structure I for this alkaloid.

Anal.—Calc. for $C_{21}H_{23}NO_6$: C, 65.45; H, 5.97; N, 3.64. Found: C, 65.58; H, 6.02; N, 3.78.

The electron fragmentation pattern of this compound (Scheme I) was in agreement with the suggested structure. This pattern was similar to that of mecambridine (18). The spectral data of this alkaloid (I) were identical with those of demethylmecambridine as synthesized recently (19).

[2] P. Goldblat, Missouri Botanical Garden, St. Louis, Mo., personal communication.

[3] Melting points were taken with a Koffler hot-stage microscope and are uncorrected. UV spectra were recorded on a Varian Techtron 635 instrument. NMR spectra were taken with a Varian A60A instrument, using tetramethylsilane as the internal standard. Mass spectra were recorded on a CH5 spectrometer. IR spectra were obtained with a Leitz model III spectrograph.

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Antitumor Agents XV: Deoxyelephantopin, an Antitumor Principle from *Elephantopus carolinianus* Willd.

KUO-HSIUNG LEE ˣ, CAROLE M. COWHERD *, and MacARTHUR T. WOLO

Abstract □ The active principle responsible for the significant inhibitory activity against the Walker 256 carcinosarcoma (ascites) in rats, isolated from the chloroform extract of the whole plant of *Elephantopus carolinianus* Willd., was characterized as deoxyelephantopin, a sesquiterpene lactone.

Keyphrases □ *Elephantopus carolinianus* Willd.—isolation and structure identification of deoxyelephantopin, antitumor activity □ Deoxyelephantopin—isolation from *Elephantopus carolinianus* Willd., structure identification, antitumor activity □ Antitumor agents, potential—deoxyelephantopin, major constituent of *Elephantopus carolinianus* Willd. □ Structure–activity relationships—sesquiterpene lactones (deoxyelephantopin) as antitumor agents

As a result of the continuing search for *Elephantopus* principles having new and novel potential antitumor agents (1, 2)[1], the chloroform extract of the whole plant of *Elephantopus carolinianus* Willd. (Compositae) was found to show significant inhibitory activity against the Walker 256 carcinosarcoma (ascites) in rats[2]. The extraction of the active principles was carried out according to an exact procedure described in the literature (4). A preliminary examination of the chloroform extract revealed that the antitumor activity was associated with the presence of the terpene-like constituents. Fractionation, characterization, and screening[2] of the isolated compound led to the conclusion that this major active principle was the germacranolide dilactone, deoxyelephantopin (I).

DISCUSSION

Deoxyelephantopin (I), the antitumor principle, was isolated in 0.15% yield as colorless needles from the chloroform eluate. Deoxyelephantopin, mp >320° (sintered at 198–200°)[3], has the empirical formula $C_{19}H_{20}O_6$ as determined by high-resolution mass spectrometry[4]. It shows IR bands in chloroform[5] at 1760 (double strength) and 1650 cm^{-1} and a pair of low field doublets in the NMR spectrum ($CDCl_3$)[6] at δ 6.22 (1H, d, J = 3.5 Hz, H-13) and 5.64 (1H, d, J = 3.5 Hz, H-13)[7], characteristic of an α-methylene-γ-lactone ring system.

An IR band at 1710 cm^{-1} and a characteristic base peak in the mass spectrum of I at m/e 69 suggested the presence of a methacrylate ester side chain, and the NMR signals at δ 1.93 (3H, t, J = 1.0 Hz, H-18), 5.66 (1H, t, J = 1.0 Hz, H-19)[7], and 6.14 (1H, t, J = 1.0 Hz, H-19), which are comparable with those found in molephantin (1) and phantomolin (2), established the presence of this structural feature. The NMR spectrum of I showed, in addition to the well-defined one-proton doublet of doublets (δ 5.13, J = 10.0

[1] See K. H. Lee, T. Ibuka, H. C. Huang, and D. L. Harris, *J. Pharm. Sci.*, **64**, 1077(1975).

[2] *In vivo* antitumor activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, using a literature method (3). Deoxyelephantopin showed significant inhibitory activity against the Walker 256 (ascites) carcinosarcoma in rats at 226% T/C at the 2.5-mg/kg level. A compound is active if it exhibits a T/C of ≥125% (3).

[3] Reference 5 reported mp 198–200°.

[4] The authors thank Dr. David Rosenthal and Mr. Fred Williams of the Research Triangle Center for Mass Spectrometry for mass spectral data.

[5] This IR absorption band shifted to 1755 and 1738 cm^{-1} in mineral oil. Reference 5 reported 1766 and 1747 cm^{-1} in KBr.

[6] The authors thank Dr. David L. Harris of the Department of Chemistry, University of North Carolina at Chapel Hill, for NMR measurements. The model XL-100 used for the NMR measurements was purchased from grants from the National Science Foundation and the National Institutes of Health to the Department of Chemistry, University of North Carolina at Chapel Hill.

[7] Partially overlapped with H-19. Reference 5 reported H-13 and H-19 as a multiplet at δ 5.64.