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THE IDENTITY OF CHILENINONE WITH BERBERRUBINE. THE PROBLEM OF TRUE NATURAL PRODUCTS VS. ARTIFACTS OF ISOLATION

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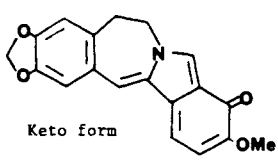
ABSTRACT.—Chileninone corresponds to berberrubine (**2**). Silica gel column chromatography of berberine-chloroform (**4**) led to the formation of small amounts of oxyberberine (**5**), berberrubine (**2**), and polyberbine (**6**). Column chromatography of palmatine-chloroform (**4a**) furnished some saulatine (**23a**); and column chromatography of (+)-boldine methiodide (**16**) gave rise to (+)-boldine free base and boldinemethine (**17**). Puntarenine (**23**), saulatine (**23a**), and magallanesine (**25**) must be artifacts, formed on the chromatographic column.

While in the process of working with some isoindolobenzazepines, it came to our attention that a sample of the recently described tetracyclic compound chileninone, obtained as a result of a study of *Berberis actinacantha, Berberis darwinii,* and *Berberis valdiviana* (Berberidaceae), and which had been assigned isoindolobenzazepine structure **1** (1), was identical in all respects with an authentic sample of the known isomeric species berberrubine (**2**). It was fully realized at the time the chemistry of chileninone was being investigated that the identity of any isoindolobenzazepine could be assigned with certainty only after proper comparison with the corresponding protoberberine, especially since the nmr spectra in the two series are so similar. In the specific instance above, however, a comparison involving, in particular, a mass spectral study of the reduction products of the two compounds was not properly carried out in the laboratory, thus accounting for the error.

Because berberrubine (**2**) and chileninone are identical compounds and because berberrubine was previously known, the name chileninone should not be used further to describe this material. Additionally, the reported (1) $NaBH_4$ reduction product of chileninone corresponds to tetrahydroberberrubine.

This misidentification had some further ramifications. While searching the literature for berberrubine to ascertain some of its physical properties, we were struck by the fact that, although berberrubine had been found in a variety of plant families, including the Berberidaceae (2,3). Menispermaceae (4,5), and Ranunculaceae (6-9), it was usually accompanied by the very common alkaloid berberine (**3**). There was a possibility, therefore, that berberrubine (**2**) could have been formed from berberine (**3**) during the isolation process.



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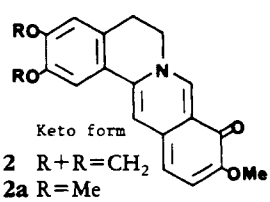
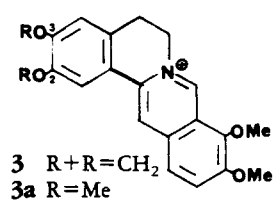


2 R+R=CH_2
2a R=Me



3 R+R=CH_2
3a R=Me

In order to test the above hypothesis, we had to resort to a simple experiment. A sample of berberine (**3**) chloride was first treated with NH_4OH and $CHCl_3$ to obtain berberine-chloroform (**4**). Since $CHCl_3$ and NH_4OH are usually used during alkaloid isolation, the aforementioned procedure does not depart appreciably from standard al-

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kaloid isolation technique. The berberine-chloroform was then placed on a silica gel column of the type usually used in alkaloid isolation, elution being with $CHCl_3$/MeOH. Besides the expected berberine and berberine-chloroform, three products were identified, although in small amounts. The first proved to be the known oxyberberine (**5**), indicating that oxidation had occurred on the column. Just like berberrubine (**2**), oxyberberine has been reported as a natural product in several botanical families including the Berberidaceae, Rutaceae, and Menispermaceae.[2]

The second was berberrubine (**2**), which is clearly the product of *O*-demethylation. This result is reminiscent of a recently published report where it was clearly shown that certain *N*-demethylations can also occur extremely readily upon chromatography. Specifically, the *N*-methylated oxoaporphinium salts uthongine and thailandine are partially converted by simple tlc on silica gel into the oxoaporphine-free bases oxocrebanine and oxostephanine, respectively (11).

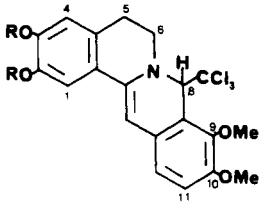
The third product from the column was the most unexpected inasmuch as it corresponded to polyberbine (**6**), reported to be present in extracts of *B. valdiviana* (12). Polyberbine is usually classified as a pseudobenzylisoquinoline. All pseudobenzylisoquinolines possess a benzylisoquinoline skeleton with three oxygenated substituents present in the lower aromatic ring at positions 2′, 3′, and 4′.

From an historical standpoint, the first pseudobenzylisoquinoline to be isolated was polycarpine (**7**) (13). Then, and in fairly rapid succession, rugosinone (**8**) (14,15), dihydrorugosinone (**9**) (16), (−)-ledecorine (**10**) (17), and taxilamine (**11**) (18) were described, as well as polyberbine (**6**). Pseudobenzylisoquinolines have been obtained as a result of studies of various extracts from members of the Annonaceae, Berberidaceae, Fumariaceae, and Ranunculaceae. Those pseudobenzylisoquinolines that are not formylated on nitrogen can, of course, be derived from hydrolysis of the formyl function—a transformation that is usually followed by oxidation.

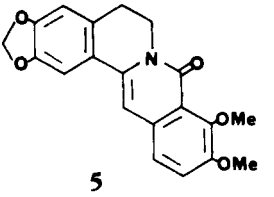
A valid question at this stage, therefore, concerns the true identity of berberrubine (**2**), oxyberberine (**5**), and the pseudobenzylisoquinolines. Are they true alkaloids or artifacts of isolation? It is difficult to answer this question with finality, but it is probable that most of them are true alkaloids. In the case of berberrubine (**2**), *O*-demethylation could presumably occur in vivo at least as readily as it could in vitro. Similarly, oxidation of a protoberberine base to an oxyprotoberberine could well be a biogenetic process. It should be pointed out in this respect that the optically active lactam (−)-8-oxotetrahydropalmatine (**12**) has recently been obtained from *Anamirta cocculus* (Menispermaceae) (19). This compound is obviously an alkaloid, and its in vivo oxidation with aromatization of ring C would lead to an oxyprotoberberine.

The problem posed by the pseudobenzylisoquinolines is somewhat more complex. In the first place, ledecorine (**10**) is an *N*-methylated and optically active species, which points towards a natural origin. Secondly, it has been recognized that a protoberberinium salt is transformed into its corresponding pseudobenzylisoquinoline by an oxidative rearrangement involving an aryl migration which may be duplicated in the laboratory, as in the known in vitro conversion of berberine (**3**) into polyberbine (**6**), and palmatine (**3a**) into polycarpine (**7**) (20). What is particularly significant is that this oxidative rearrangement has its counterpart among the indole alkaloids. Thus, the dimeric indole alkaloids catharine and catharinine have been found in a variety of *Catharanthus* species (Apocynaceae) and are structurally related to the important and accompanying antitumor alkaloid vinblastine (21-23). Catharine and catharinine bear an *N*-formyl group, so that their biogenesis probably proceeds from vinblastine by an oxidative rearrangement paralleling that involved in the transformation of berberine (**3**)

[2]For a partial listing of oxyberberine-derived plant materials, see Jeffs (10).



4 R+R=CH_2
4a R=Me



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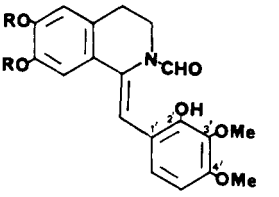
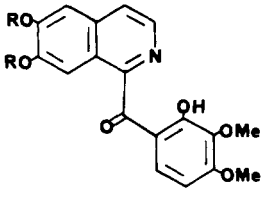
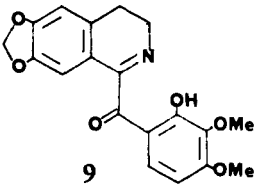


6 R+R=CH_2
7 R=Me



8 R+R=CH_2
11 R=Me



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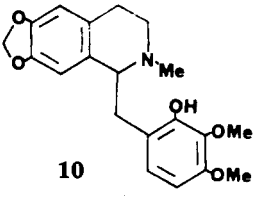

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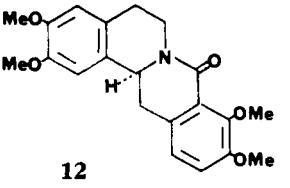

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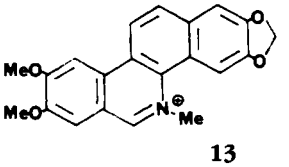
into polyberbine (**6**), and palmatine (**3a**) into polycarpine (**7**). In the indole alkaloid series, however, the migrating group is an alkyl rather than an aryl group (24).

The most telling argument in favor of a natural oxidative rearrangement concerns the triad of benzophenanthridine alkaloids represented by nitidine (**13**), isoarnottianamide (**14**), and chelilutine (**15**).[3] Isoarnottianamide (**14**) is probably formed from nitidine (**13**) or a close analog, and sequential cyclization of the former followed by *O*-methylation would provide chelilutine (**15**). Obviously, no in vitro *O*-methylation of a phenolic function could readily occur on a chromatographic column during isolation, so that chelilutine and, hence, isoarnottianamide are alkaloids. It is quite likely, therefore, that pseudobenzylisoquinolines are natural products.

At this stage, we wanted to find out just how extensive were the possible chemical transformations that could take place on a chromatographic column. The aporphine quaternary salt (+)-boldine methiodide (**16**) was, therefore, placed on a silica gel column, and eluted with $CHCl_3$/MeOH. Besides the starting material, small amounts of boldine free-base as well as boldinemethine (**17**) were obtained. Again, we have here a case of *N*-demethylation; but even more importantly, we have a Hofmann β-elimination. Does this result then mean that the phenanthrene alkaloids that are similar to boldinemethine in their carbon skeleton are artifacts?[4]



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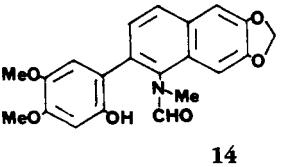

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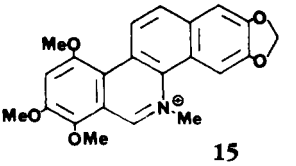

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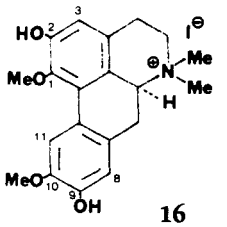
[3]For a discussion of the chemistry of these alkaloids, see Shamma and Moniot (25).

[4]For a listing of aporphines and phenanthrenes, see Guinaudeau *et al.* (26).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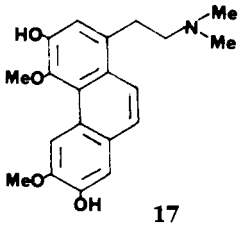
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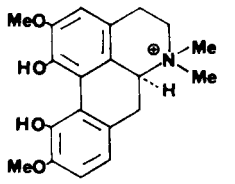
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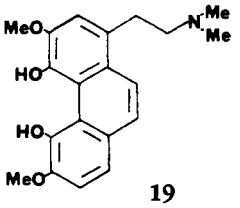
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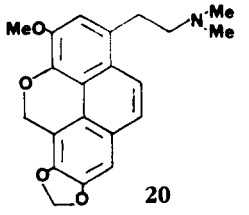
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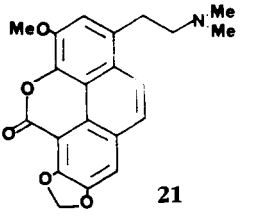
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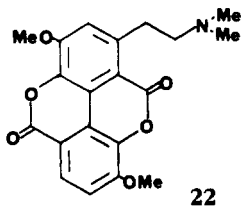
In order to attempt to answer this question, it is appropriate to point out that all the classical-type phenanthrene alkaloids reported so far in the literature (namely, argentinine, atherosperminine, methoxyatherosperminine, uvariopsine, noruvariopsine, uvariopsamine, uvariopsamine *N*-oxide, thalicthuberine, 8-methoxyuvariopsine, noratherosperminine, methoxyatherosperminine *N*-oxide, and atherosperminine *N*-oxide) are unsubstituted at C-11 (aporphine numbering system) (26). In line with this observation is the fact that, while the alkaloidal salt (+)-magnoflorine (**18**) may be the most widely occurring of the aporphines (26), its Hofmann elimination product, magnoflorinemethine (**19**), has so far been unreported as a natural product. A likely reason for this trend is that the energy barrier required to proceed from an *N*-methoaporphinium salt to its Hofmann methine is less when C-11 is unsubstituted. In other words, the steric interaction between a C-1 and a C-11 substituent on an aporphine makes it more difficult to attain the near planarity required in phenanthrene formation.

There are actually some exceptions to the above observation, but they are the exceptions which, in a sense, underline the trend. The known bases thaliglucine (**20**), thaliglucinone (**21**), and thalflavidine are phenanthrenes that incorporate substituents at C-1 and C-11. However, these substituents are in the form of a methylenoxy or a δ-lactone bridge, which does not hinder the planarity of the phenanthrene skeleton (26).

It would be incorrect to conclude from these observations that phenanthrenes are artifacts formed from aporphine quaternary salts during the isolation process, whenever C-11 is unsubstituted. In particular, the dilactonic alkaloid taspine (**22**) (26) is most probably formed from magnoflorine (**18**) via its methine base, so that nature can produce phenanthrenes even from aporphine salts substituted at C-11. Again we have here, therefore, a case where what happens on the chromatographic column upon Hofmann β-elimination of an *N*-methoaporphinium salt seems to parallel to some extent the biogenetic sequence.

Subtle cases of artifact formation can arise whenever $CHCl_3$ is used on a silica gel column. We had noticed from the literature (5) that the occurrence of palmatrubine (**2a**) is much less common than that of its methylenedioxy analog berberrubine (**2**). We therefore decided to study the behavior of palmatine-chloroform (**4a**) on a silica gel column which was eluted with $CHCl_3$/MeOH. Surprisingly, besides starting material and palmatine (**3a**), we obtained the ketolactam saulatine (**23a**), which had been first reported in 1984 as a result of work on *Abuta bullata* (Menispermaceae) (27). One year before that time, two independent studies had found the related ketolactam puntarenine (**23**) in the course of investigations of the contents of *B. actinacantha* (28,29).



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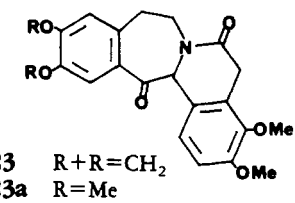
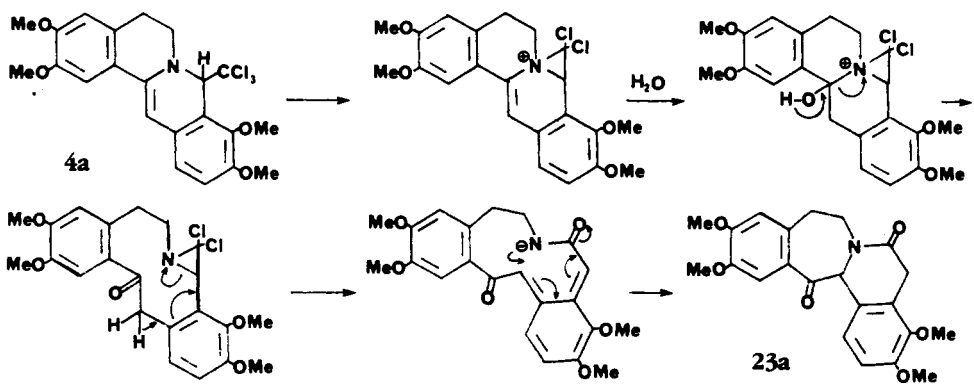


23 R+R=CH_2
23a R=Me

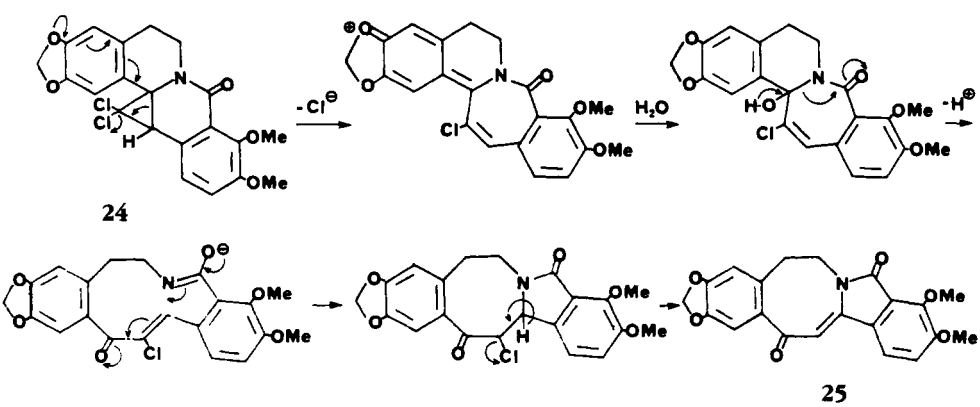
Since we had started our chromatographic study of palmatine-chloroform with clean material and since we had never previously isolated saulatine in our laboratory, it can only be concluded that saulatine (**23a**) and, by extension, puntarenine (**23**) are artifacts. Saulatine (**23a**) could have been formed on the column by the mechanism indicated in Scheme 1.



SCHEME 1. Saulatine formation

Similarly, the ketolactam magallanesine (**25**), obtained as part of a study of *B. darwinii* (30), can be formed as shown in Scheme 2 through the intermediacy of the dichloro adduct **24** derived from oxyberberine (**5**) (31). Saulatine (**23a**), puntarenine (**23**), and magallanesine (**25**) are thus interesting instances of artifacts formed from $CHCl_3$, even though they contain no chlorine.

It is difficult to rationalize the difference in reactivity recorded here between berberine (**3**) on the one hand, and palmatine (**3a**) on the other. It should be noted, however, that the methylenedioxy substituent on ring A of berberine is a better electron



SCHEME 2. Magallanesine formation

donor through resonance than the more hindered methoxyl groups at C-2 and C-3 of palmatine.

We have seen how *N*-demethylation, *O*-demethylation, oxidation, oxidation with rearrangement, Hofmann β-elimination, and ring expansion can occur on a chromatographic column. It is, therefore, incorrect to assume that what is placed on a chromatographic column necessarily corresponds to what actually comes out of it. This is particularly true in the case of natural product isolation, where one usually starts with large amounts of plant extracts that are chromatographed on a column over a period of several weeks, if not months. It is only necessary to consider the situation prevailing under such circumstances. In the first place, silica gel is an active surface where O_2 and H_2O from the atmosphere are adsorbed. Secondly, over several weeks or months, the column is continuously flushed with solvents which are, themselves, saturated or nearly saturated with O_2 and H_2O.

There is no single absolute procedure that can be offered to prevent or to detect artifact formation during the isolation-purification process of compounds from natural origin. In the case of the *N*-methooxoaporphinium salts uthongine and thailandine, some *N*-demethylation occurred even on the tlc plate (11).

However, the following remarks may be relevant to natural product isolation: (a) Some of the original plant or animal extracts should be kept behind to compare with material coming out of the chromatographic column. (b) If at all possible, column chromatography should not be allowed to take place over long periods of time. Similarly, no compound should be allowed to remain for a long time on a tlc plate. (c) It may be desirable in some instances to replace $CHCl_3$ with CH_2Cl_2. (d) Analysis of extracts by hplc or related techniques would be highly desirable in certain cases. (e) The main compound found in an extract could be passed slowly through a chromatographic column to determine any chemical changes it may undergo in the process.

The discussion above has been limited mainly to the isoquinoline alkaloids simply because of our interest in this area. Nevertheless, these conclusions may apply equally well to other realms of natural product chemistry.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Berberine chloride was purchased from Mehta Pharmaceuticals, G.T. Road, Chheharta, Amritsar, India. Column chromatography was on Merck Silica Gel 60. Tlc was on Merck Silica Gel 60 F-254 glass plates with 0.25 or 0.5 mm thickness. All ^{1}H-nmr spectra were obtained in $CDCl_3$ solution either at 200 or at 360 MHz. The compounds obtained by chromatography were identified through their nmr, mass, uv, and ir spectra.

BERBERINE-CHLOROFORM (**4**).—Berberine chloride (2 g) was stirred with $CHCl_3$ (100 ml) and NH_4OH (4 ml) overnight. Any precipitate of berberine was filtered off. The $CHCl_3$ solution was separated and the solvent evaporated at reduced pressure. The brown residue crystallized from $CHCl_3$; mp 180-182° (dec.). The compound is best described by its nmr spectrum: δ 2.74, 3.35, 3.70, 3.87 (1 H×4, m, H-5 and H-6), 3.88 and 3.94 (3 H×2, s, 9-OCH_3 and 10-OCH_3), 5.64 (1 H, s, H-8), 5.94 (2 H, dd, OCH_2O), 6.10 (1 H, s, H-13), 6.62 (1 H, s, H-4), 6.88 (1 H, d, H-12, J=8.4 Hz), 6.98 (1 H, d, H-11, J=8.4 Hz), 7.17 (1 H, s, H-1).

COLUMN CHROMATOGRAPHY OF BERBERINE-CHLOROFORM (**4**).—Berberine-chloroform (200 mg) in $CHCl_3$-MeOH (99:1 v/v) (4 ml) was placed on a chromatographic column (1.5 cm diameter) that had been packed with silica gel (20 g) to a height of about 22 cm. The initial elution solvent was $CHCl_3$-MeOH (99:1), with the percentage of MeOH slowly allowed to increase with time. Fractions of approximately 20 ml each were slowly collected over a period of 1 week. The contents of each fraction were checked by tlc, and like fractions were amalgamated. Final purification was by tlc. The following compounds were identified, with the approximate elution times indicated: berberine-chloroform (**4**) (85 mg), 35 h; polyberbine (**8**) (1.3 mg), 37 h; oxyberberine (**5**) (3 mg), 37 h; berberrubine (**2**) (2.1 mg), 67 h; berberine (>31 mg), 117 h. Several very minor spots were also observed.

PALMATINE-CHLOROFORM (**4a**).—This was prepared from palmatine chloride, $CHCl_3$, and

NH_4OH as indicated for berberine-chloroform, mp 188-190° (dec.) (32); nmr δ 2.75, 3.42, 3.75, 3.93 (1 H×4, m, H-5 and H-6); 3.90, 3.91, 3.95, 3.96 (3 H×4, s, four OCH_3), 5.65 (1 H, s, H-8), 6.16 (1 H, s, H-13), 6.64 (1 H, s, H-4), 6.90 (1 H, d, J=8.4 Hz, H-12), 6.99 (1 H, d, J=8.4 Hz, H-11), 7.19 (1 H, s, H-1).

COLUMN CHROMATOGRAPHY OF PALMATINE-CHLOROFORM (**4a**).—Palmatine-chloroform (30 mg) in $CHCl_3$-MeOH (99:1) (3 ml) was placed on a chromatographic column (1 cm diameter) that had been packed with silica gel (15 g) to a height of approximately 31 cm. The initial elution solvent was $CHCl_3$-MeOH (99:1), with the percentage of MeOH slowly allowed to increase. Once the palmatine-chloroform had been completely deposited on the silica gel, the column was stopped for 3 days. Elution was then resumed. The compounds identified were palmatine-chloroform (10 mg) and saulatine (**23a**) (7 mg). Several very minor spots were also observed.

COLUMN CHROMATOGRAPHY OF (+)-BOLDINE METHIODIDE (**16**).—The aporphine salt (250 mg) in $CHCl_3$-MeOH (91:9) was placed on a chromatographic column (1.5 cm diameter) that had been packed with silica gel (25 g) to a height of about 27 cm. Elution was with $CHCl_3$-MeOH (91:9). Once the salt had been firmly placed on the silica gel, the chromatography was stopped for 30 h. Elution was then resumed, and 20-ml fractions were collected. The polarity of the solvent was slowly increased to $CHCl_3$-MeOH (75:25) over a period of 1 week. Major fractions collected consisted of (+)-boldine (3 mg), boldinemethine (**17**) (16 mg), and boldine methiodide (>100 mg).

BOLDINEMETHINE (**17**).—$C_{20}H_{23}NO_4$; amorphous; nmr δ 2.28 (6 H, s, $N(CH_3)_2$), 2.58 (2 H, m, α-CH_2), 3.10 (2 H, m, β-CH_2), 3.71 (3 H, s, OCH_3), 3.91 (3 H, s, OCH_3), 6.94 (1 H, s, ArH), 7.07 (1 H, s, ArH), 7.31 (1 H, d, J=9.1 Hz, H-9 or H-10), 7.52 (1 H, d, J=9.1 Hz, H-9 or H-10), 8.94 (1 H, s, H-5); uv λ max (MeOH) 263, 282 sh, 305, 318, 348 nm (log ε 4.48, 4.11, 3.75, 3.07); ms m/z 341 (M^+) (1), 283 (1), 240 (1), 58 (100).

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