Phytochemistry, Vol. 26, No. 11, pp. 2991–2994, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
© 1987 Pergamon Journals Ltd.

BIOTRANSFORMATION OF ISOPAPAVERINE BY *SILENE ALBA* CELL SUSPENSIONS

HÉLÈNE CHRISTINAKI, FRANÇOISE BISTER-MIEL*, ABDERAHMANE HAMMOUMI*, MICHELE BURY*, JEAN-LOUIS GUIGNARD* and CLAUDE VIEL

Pharmacochimie des substances naturelles et de synthèse, U.A. 496 C. N. R. S;* Botanique et Phytochimie, University of Paris-Sud, Faculty of Pharmacy, 5 rue J. B. Clément, 92296 Châtenay Malabry Cédex, France

(*Revised received* 10 *March* 1987)

Key Word Index—*Silene alba*; Caryophyllaceae; cell suspensions; isopapaverine; 4-benzyl isoquinolines; biotransformations; syntheses; structural analysis.

Abstract—*Silene alba* cell suspension was supplied with isopapaverine (methanesulphonate), a papaverine analogue. The mechanisms of biotransformation were identical to those of papaverine, i.e. formation of four monohydroxy compounds resulting from the *O*-demethylation of the isopapaverine molecule, benzylic oxidation with formation of isopapaveraldine and isopapaverine *N*-oxide formation. The results obtained with papaverine and isopapaverine show that the oxidative enzymes are not specific for a definite benzylisoquinoline molecular structure.

INTRODUCTION

The biotransformation of papaverine (1) by *Silene alba* Miller E. H. L. Krause cell suspension cultures has been reported in preceding papers [1–3]. The metabolites produced are formed by three types of reaction: *O*-demethylation, in which the 6-and 4'-monodemethyl papaverines are predominant; oxidation of the benzyl group to a benzoyl group with formation of papaveraldine; *N*-oxidation of the nitrogen atom with *N*-oxide formation [1–3].

This paper presents the structural elucidation of the metabolites of isopapaverine (2) produced in *S. alba* cell suspension cultures. This substrate [4] is a structural analogue of papaverine which has the same conformation, pKa and chemical behaviour [5, 6]. However, it differs in the position of the benzyl group which occurs at C-4 in isopapaverine and at C-1 in papaverine.

Isopapaverine has been chosen in order to find whether the same type of metabolites are formed and also to determine the specificity of the enzymatic systems involved in the biotransformations.

RESULTS AND DISCUSSION

Estimation of toxicity

Isopapaverine methanesulphonate toxicity on *S. alba* cell suspension cultures was studied in media containing 0–1000 mg/l of substrate (Fig. 1). A slight stimulation of growth was observed for low concentrations of isopapaverine methanesulphonate, i.e. an increase of 6% in the dry weight of the cells at a concentration of 50 mg/l. It had been previously shown that the increase in environmental acidity in this range is more conducive to cell growth [7]. Therefore, the stimulative action could be explained by the influence of isopapaverine methanesulphonate in slowing the alkalinisation of the medium. At concentrations greater than 100 mg/l growth was decreased. The concentration for which the growth was reduced to 50% weight was 400 mg/l. For papaverine, this concentration is 800 mg/l [1].

Estimation of biotransformation capacity

The biotransformation capacity of *S. alba* cell suspension cultures was evaluated qualitatively at the end of the exponential growth phase. Three assays were performed with 200 mg/l and two with 400 mg/l of isopapaverine methanesulphonate. Culture medium samples were taken after seven days of growth and analysed as described

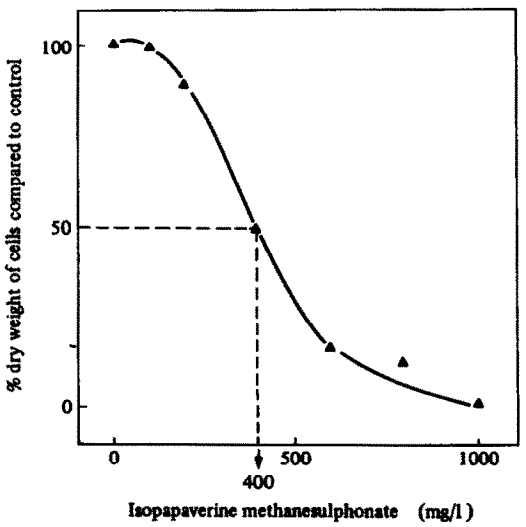


Fig. 1. Toxicity of isopapaverine methanesulphonate towards *S. alba* cell suspension.

earlier. Five metabolites and isopapaverine were detected by TLC and GC/MS.

Extracts of the cells contained insufficient amounts of metabolites to permit their visualization on TLC, but GC/MS showed the presence of two metabolites and isopapaverine.

No metabolites were detected in cells and medium after hydrochloric acid hydrolysis [1].

Structure determination of metabolites

(*a*) *From the medium.* GS/MS showed: (1) Depending on the concentration of the extract injected in the mass spectrometer, three or four spectra with similar molecular peaks (*m/z* 325) and fragmentation patterns corresponding to monodemethylated isopapaverines (**3–6**) [abundant fragments at *m/z* 325 (100%), 310, 294, 279, 151]. (2) One spectrum which corresponds to isopapaveraldine (**7**) [abundant fragments at *m/z* 353, 338, 322, 216, 188, 165 (100%). (3) Two spectra with fragmentation patterns similar to that of isopapaverine (**2**) [abundant fragments at 339 (100%), 338, 324, 308]. One corresponded to isopapaverine and the other to isopapaverine *N*-oxide (**8**) after loss of oxygen from the *N*-oxide group. The structure of compound **8** (which has a longer R_f than isopapaverine) was confirmed by co-TLC in comparing with authentic sample and by the mass spectra (GC/MS) of prepared *N*-oxide compound. It is important to note that the mass spectrum recorded by the direct introduction shows the molecular peak at *m/z* 355 (100%), but the spectrum obtained by GC/MS shows the same base peak at 339 due to the loss of oxygen of the *N*-oxide group. This behaviour is common to the heterocyclic *N*-oxides [8].

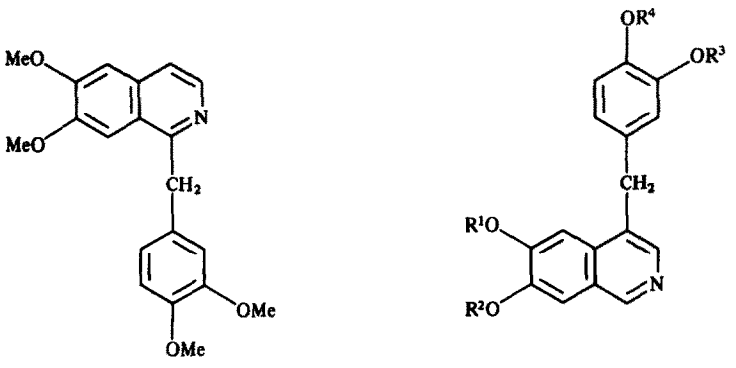
The location of the phenolic group in each of the monodemethylated isopapaverines was deduced by comparing the information obtained by GC/MS and TLC of these metabolites and the four synthetic monodemethyl isopapaverines, **3–6**. The GC/MS identification of these compounds was effected by comparison of the mass spectral fragments and the order of elution of the metabolites and synthesized compounds (R_f 7–OH < R_f 4′–OH < R_f 3′ –OH < R_f 6-OH when the demethylated isopapaverines were injected separately; R_f 7–OH = R_f 4′ –OH when the compounds were injected in a mixture). The TLC determination of the positions of the hydroxyl groups was based on the R_f values and on the different colours obtained with Gibbs' reagent.

A blue colour was obtained for compound **5**, indicating a free *para* hydroxyl group [9]; a blue-green colouration was given by **4**, a green colouration with **6** and a clear brown colour with **3**. It is interesting to note that the spots of **5** and **6** were very close together as were those of **3** and **4**. For these last compounds, the UV fluorescence on TLC is different at 366 nm: **4**, intense green; **3**, intense blue.

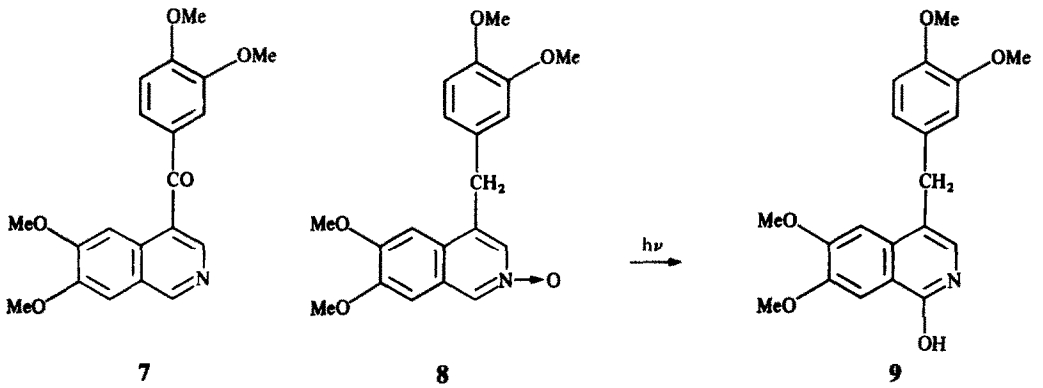
On the basis of these different results with special reference to the colour intensities of the spots, it is possible to infer that **4** is the major metabolite whereas **5** and **6** occur in lower concentration with **3** occurring in least concentration.

Concerning the *N*-oxide, no transposition to the corresponding isocarbostyril occurs, as we have observed after photoirradiation of the synthetic *N*-oxide.

(b) *From the cells.* Following the same procedures as for the medium, *N*-oxide and traces of 7-hydroxy isopapaverine were observed in addition to isopapaverine. The low concentrations of the metabolites have not permitted their



2 $R^1 = R^2 = R^3 = R^4 = Me$
3 $R^1 = H$, $R^2 = R^3 = R^4 = Me$
4 $R^1 = R^3 = R^4 = Me$, $R^2 = H$
5 $R^1 = R^2 = R^4 = Me$, $R^3 = H$
6 $R^1 = R^2 = R^3 = Me$, $R^4 = H$



quantification. No metabolite was detected after hydrolysis of cells and medium. This shows the absence of isopapaverine or metabolite complexation.

CONCLUSION

It can be stated that the accessibility of the 3' and 4'-methoxyl groups towards oxidative enzymes is similar in isopapaverine and papaverine. By contrast, the demethylation of the C-7 methoxyl group of isopapaverine occurs predominantly, whereas in the case of papaverine demethylation of the C-6 methoxyl group takes place preferentially. This fact can be explained by the conformation of the benzyl group in these two alkaloid molecules. In papaverine and isopapaverine, the benzyl group is placed towards the isoquinoleic homocycle. Therefore, in papaverine, the presence of benzyl at C-1 hinders C-7 position and the C-6 methoxyl group is easily be oxidized. In contrast, it is the C-7 methoxyl which is more accessible to oxidative enzymes in isopapaverine.

The results obtained with papaverine and isopapaverine show that the oxidative enzymatic systems are not specific for a definite benzylisoquinoleic molecular structure.

EXPERIMENTAL

Culture, substrate stability and bioassay. Cell cultures of *S. alba* were grown in Murashige and Skoog liquid medium supplemented with sucrose (30 g/l), adenine (7.4×10^{-6} M), 2,4-dichlorophenoxyacetic acid (5×10^{-6} M) and kinetin (5×10^{-6} M), as detailed in an earlier paper [10].

The stability of a 1 g/l isopapaverine methanesulphonate soln was checked under the experimental conditions used for the biotransformations, in the absence of cells. No degradation products were revealed by TLC.

Bioassays were performed by incubating cell suspensions with isopapaverine methanesulphonate, at $23 \pm 1°$, on a rotary shaker at 180 rpm under illumination of 25 μE/m/sec for 12 h per day. Seven-day-old cell suspensions were inoculated at a dilution of 1/11 into new medium with 200 mg/l of isopapaverine methanesulphonate. After seven days of growth, cell samples were collected by red. pres. sieving dried at 60° for 48 hr and then weighed.

Extraction and analysis. The culture medium and cells were separately extracted. The medium was adjusted to pH 10 with NH_4OH extracted first by EtOAc and then with CH_2Cl_2. Each organic extract was subsequently dried over anhydrous Na_2SO_4 and evapd under red. pres. The cells were frozen after their separation from the culture medium. Thawed cells were adjusted to pH 10 with NH_4OH crushed and extracted with the same organic solvents as those used for the culture medium.

Subsequently, the medium and cells were separately hydrolysed with 5% H_2SO_4, refluxed for 1 h and filtered. The acidic soln was alkalized with NH_4OH and extracted as reported for medium and cells.

The crude extracts were analysed by GC-MS (70 eV OV1 capillary column (25 m), 180° to 280°, 10°/min) and by TLC (Schleicher and Schuell silicagel plates F 1500/LS 254, 0, 25 mm thick, CH_2Cl_2–MeOH, 23:2). The compounds were visualized by UV light at 254 and 366 nm, Dragendorff's reagent (Prolabo) and Gibbs' reagent [11]. After spraying with the reagents the plates were exposed to NH_3 vapour.

Syntheses of isopapaverine and its metabolites.. Mps: heated microscope; NMR: 60 MHz with TMS as an int. stand. MS: Nermag R10–10C spectrometer. All analyses (C, H and N) were within ±0.3% of the calcd values. Only the general formulae and *M*,s are reported. The TLC procedures were similar to those of cells and medium culture extracts.

Substituted 4-benzyl isoquinolines. (a) Substituted benzylamino acetaldehyde diethylacetals. Veratrylamino acetaldehyde diethylacetal was prepared according to [4] by condensation of veratraldehyde with aminoacetaldehyde diethylacetal, then reduction by $NaBH_4$. 3- or 4- methoxy-and 4- or 3-hydroxybenzylamino acetaldehyde diethylacetals were obtained by condensation of vanillin or isovanillin with aminoacetaldehyde diethylacetal followed by hydrogenation at 3 bars on PtO_2 for 2.3 h of an ethanolic soln of the crude iminoacetals. After filtration and evaporation of the solvent under reduced pressure the aminoacetals were recrystallized from Et_2O.

6,7-Dimethoxy veratrylaminoacetaldehyde diethylacetal. Prepared by Bouvier *et al.* [4]; $E_{0,15} = 144–147°$; $n_D^{8,2°} = 1.507$; IR ν^{film} cm^{-1}: 3345 (N-H), 1590 (C=C); ^1H NMR ($CDCl_3$); δ 1, 21 (*t*, 6H, 2-Me), 1,6 (*s*, 1H,-NH), 2,76 (*d*, 2H, $-\underline{CH_2}-NH-$), 3,61 (*m*, 6H, $3 \times CH_2$), 3, 86 (*s*, 3H, –OMe) 3, 93 (*s*, 3H, –OMe), 4, 63 (*t*, 1H, –CH–) and 6, 9 (*m*, 3H, aromatic H).

3-Methoxy 4-hydroxy benzylamino acetaldehyde diethyl acetal. Yield 71%; mp 70–71°; Analysis: $C_{14}H_{23}NO_4$ (269), C, H, N; IR ν^{CHCl_3} cm^{-1}: 3560 (–OH), 3340 (N–H), 1590 (C=C); ^1H NMR ($CDCl_3$) δ 1, 43 (*t*, 6H, $2 \times CH_3$), 3, 05 (*d*, 2H, $-\underline{CH_2}-Ph$), 3, 73 (*m*, 6H, $3 \times CH_2$), 4, 41 (wide *s*, 1H, OH), 4, 9 (*t*, 1H, CH–) and 7, 05 (*m*, 3H, aromatic H).

4-Methoxy 3-hydroxy benzylamino acetaldehyde diethylacetal. Yield 68%; mp 60–61° Analysis: $C_{14}H_{23}NO_4$ (269), C, H, N; IR ν^{CHCl_3} cm^{-1}: 3560 (OH), 3340 (N–H), 1590 (C=C); ^1H NMR ($CDCl_3$) δ: 1, 21 (*t*, 6H, $2 \times CH_3$), 2, 76 (*d*, 2H, $-CH_2-Ph$), 3, 51 (*m*, 7H, $3 \times CH_2$ and $\underline{OH}$), 3, 75 (*s*, 3H, $-OCH_3$), 4, 66 (*t*, 1H, –CH) and 6, 86 (*m*, 3H, aromatic H).

(b) Substituted 4-benzyl isoquinolines. Prepared according to [4]: 2, 12 mmol of substituted benzylamino acetaldehyde diethylacetal were added dropwise to 22 ml HCl with stirring. After a few minutes a soln of 2.12 mmol of the corresponding benzaldehyde in 50 ml EtOH was added dropwise. The reaction mixture was heated at reflux for 30 min. After cooling, 150 ml of H_2O were added. The aq. soln was extracted (× 2) with toluene to remove the unreacted benzaldehyde, then alkalized with 30% NaOH except for the synthesis of the 4-benzyl isoquinolines, in which the alkalization was effected with NH_4OH to avoid phenate formation. The aq. solution was extracted (× 2) with $CHCl_3$. The combined extracts were washed with H_2O to neutrality, dried over Na_2SO_4 and evapd under red. pres.

Methanesulphonate salts were obtained by exact neutralization of a base methanolic soln with methanesulphonic acid and then recrystallization from MeOH.

6,7-Dimethoxy 4-(3',4'-dimethoxy benzyl) isoquinoline (isopapaverine) (2). Recrystallized from EtOH; Yield 31%; mp 133–134° [(4), 133–134°]; R_f 0, 7; Analysis: $C_{20}H_{21}NO_4$ (339), C, H, N; IR ν^{KBr} cm^{-1}: 1615 (C=N), 1590 (C=C); ^1H NMR (DMSO-d_6) δ 3.36 (*s*, 3H, –OMe), 3, 68 (*s*, 3H, OMe), 3, 9 (*s*, 6*H*, $2 \times$ OMe), 4, 23 (*s*, 2H–CH_2), 6, 75 (*s*, 2H, H of benzyl nucleus), 6, 93 (*s*, 1H, H of benzyl nucleus), 7, 26 (*s*, 1H, H-5) 7, 4 (*s*, 1H, H-8), 8, 16 (*s*, 1H, H-3), 8, 87 (*s*, 1H, H-1); MS *m/z* (rel. int): 340 $[M+1]^+$ (37), 339 $[M]^+$ (100), 338 $[M-1]^+$ (18), 324 $[M-Me]^+$ (16), 308 $[M-MeO]^+$ (15), 293 $[M-(MeO+Me)](7)$, 202 $[M-(MeO)_2\ C_6H_3)]^+$ (8); methanesulphonate, mp 160–161°.

7-Methoxy 6-hydroxy 4-(3',4'-dimethoxy benzyl)isoquinoline (3). Recrystallized from MeOH–$CHCl_3$ (1:3) Yield 54%; mp 215–217°; R_f 0.56; Analysis: $C_{19}H_{19}NO_4$ (325), C, H, N; IR ν^{KBr} cm^{-1}: 3600 (OH); 1615 (C=N); 1590 (C=C); ^1H NMR (DMSO-d_6) δ 3, 66 (*s*, 6H, $2 \times$ OMe), 3, 91 (*s*, 3H, OMe), 4, 16 (*s*, 2H, $-CH_2$), 6, 68 (*s*, 1H, H of benzyl nucleus), 6, 75 (*s*, 1H, H of

benzyl nucleus), 6, 9 (*s*, 1H, H of benzyl nucleus), 7, 21 (*s*, 1H, H-5), 7, 43 (*s*, 1H, H-8), 8, 15 (*s*, 1H, H-3), 8, 88 (*s*, 1H, H-1); MS *m/z* (rel. int.): 326 $[M+1]^+$ (23), 325 $[M]^+$ (100), 324 $[M-1]^+$ (23), 310 $[M-Me]^+$ (22), 308 $[M-OH]^+$ (4, 2), 294 $[M-OMe]^+$ (14), 279 $[M-(MeO+Me)]^+$ (9), 151 $[(MeO)_2\ C_6H_3CH_2]^+$ (4).

6-Methoxy 7-hydroxy 4-(3',4'-dimethoxybenzyl) isoquinoline (**4**). Recrystallized from MeOH–$CHCl_3$ (1:4). Yield 56%, mp 210–211° R_f 0.58; Analysis: $C_{19}H_{19}NO_4$ (325), C, H, N; IR ν^{KBr} cm^{-1}: 3600 (–OH); 1635 (C=N): 1600 (C=C); ^{1}H NMR (DMSO-d_6), δ3, 71 (*s*, 6H, 2 × OMe) 3, 9 (*s*, 3H, OMe), 4, 26 (*s*, 2H, $-CH_2$), 6, 83 (*s*, 2H, H of benzyl nucleus), 7, 03 (*s*, 1H, H of benzyl nucleus) 7, 31 (*s*, 1H, H-5), 7. 36 (*s*, 1H, H-8), 8, 2 (*s*, 1H, H-3), 8.86 (*s*, 1H, H-1); MS *m/z* (rel. int.): 326 $[M+1]^+$ (37), 325 $[M]^+$ (100), 324 $[M-2]^+$ (24), 310 $[M-Me]^+$ (30), 308 $[M-OH]^+$ (6), 294 $[M-OMe]^+$ (17), 279 $[M-(MeO+Me)]^+$ (13), 188 $[M-(MeO)_2\ C_6H_3]^+$ (6), 151 $[(MeO)_2\ C_6H_3\ CH_2)]^+$ (9).

6,7-Dimethoxy 4-(4'-methoxy 3'-hydroxy benzyl) isoquinoline (**5**). Recrystallized from MeOH–$CHCl_3$ (1:9); Yield 43%; mp 220–222°; R_f 0.62; Analysis: $C_{19}H_{19}NO_4$ (325), C, H, N; IR ν^{KBr} cm^{-1}: 3566 (OH), 1625 (C=N), 1580 (C=C); ^{1}H NMR (DMSO-d_6) δ: 3, 71 (*s*, 3H, OMe) 3, 88 (*s*, 3H, OMe), 3, 93 (*s*, 3H, OMe), 4, 23 (*s*, 2H, $-CH_2$), 6, 78 (*m*, 3H H of benzyl nucleus), 7, 33 (*s*, 1H, H-5), 7, 5 (*s*, 1H, H-8), 8, 26 (*s*, 1H, H-3), 8, 99 (*s*, 1H, H-1); MS *m/z* (rel. int.): 326 $[M+1]^+$ (23), 325 $[M]^+$ (100), 324 $[M-1]^+$ (78), 310 $[M-Me]^+$ (22), 309 $[M-(Me+H)]^+$ (9), 308 $[M-OH]^+$ (4), 294 $[M-OMe]^+$ (8), 137 $[(MeO)\ OH\ C_6H_3CH_2]^+$ (6).

6,7-Dimethoxy 4-(3'-methoxy 4'-hydroxy benzyl) isoquinoline (**6**). Recrystallized from MeOH–$CHCl_3$ (1:3). Yield 34%; mp 218–220°; R_f 0, 63; Analysis: $C_{19}H_{19}NO_4$ (325), C, H, N; IR ν^{KBr} cm^{-1}: 3590 (OH), 1620 (C=N), 1590 (C=C); ^{1}H NMR (DMSO-d_6) δ: 3, 7 (*s*, 3H, OCH_3), 3, 86 (*s*, 3H, OCH_3), 3, 9 (*s*, 3H, OCH_3), 4, 21 (*s*, 2H, $-CH_2$), 6, 83 (*s*, 2H, H of benzyl nucleus), 6, 95 (*s*, 1H, H of benzyl nucleus), 7, 33 (*s*, 1H, H-5), 7, 46 (*s*, 1H, H-8), 8, 23 (*s*, 1H, H-3), 8, 89 (*s*, 1H, H-1); MS, *m/z* (rel. int.): 326 $[M+1]^+$ (32), 325 $[M]^+$ (100), 324 $[M-1]^+$ (36), 310 $[M-Me]^+$ (21), 309 $[M-(Me+H)]^+$ (11) 308 $[M-OH]^+$ (5), 294 $[M-OMe]^+$ (11), 202 $[M-(MeO)\ OH\ C_6H_3]^+$ (5), 137 $[(MeO)\ OH\ C_6H_3CH_2]^+$ (5).

6,7-Dimethoxy 4-(3',4'-dimethoxy benzyl)isoquinoline N-oxide (isopapaverine N-oxide) (**8**). 309 mg (0.91 mmol) of isopapaverine and 237 mg (1.36 mmol) of *meta*-chloroperbenzoic acid (70%) were dissolved in 20 ml of $CHCl_3$ and the soln stirred at room temp. The reaction was followed by TLC. After 28 hr the soln was alkalinized with 10% NaOH, then extracted with $CHCl_3$. The organic layer was washed with H_2O to neutrality, dried over Na_2SO_4 and evapd under red. pres. The crude product was recrystallized from EtOH; Yield 92%; mp 213–215°; R_f: 0.47; Analysis: $C_{20}H_{21}NO_5$ (355), C, H, N; IR ν^{KBr} cm^{-1}: 1620 (C=N), 1590 (C=C), 1250 (N → O); ^{1}H NMR ($CDCl_3$) δ3.78 (*s*, 3H, OMe), 3.83 (*s*, 3H, OMe), 3.9 (*s*, 3H, OMe), 4, 03 (*s*, 3H, OMe), 4, 2 (*s*, 2H, $-CH_2$), 6, 71 (*s*, 1H, H of benzyl nucleus), 6, 78 (*s*, 2H, H of benzyl nucleus), 6, 96 (*s*, 1H, H-5), 7, 11 (*s*, 1H, H-8), 7, 9 (*s*, 1H, H-3), 8, 56 (*s*, 1H, H-2); MS (direct inlet) *m/z* (rel. int.); 356 $[M+1]^+$ (34), 355 $[M]^+$ (100) 354 $[M-1]^+$ (24), 340 $[M-Me]^+$ (10), 339 $[M-O]^+$ (27), 338 $[M-(O+H)]^+$ (22), 324 $[M-(O+Me)]^+$ (13), 322 (10) 308 $[M-(O+MeO)]^+$ (10), 294 $[M-(O+CH_2O+Me)]^+$ (6), 151 $[(MeO)_2\ C_6H_3\ CH_2]^+$ (23): GC-MS, 340 $[M+1]^+$ (24), 339 $[M]^+$ (100), 338 $[M-1]^+$ (24), 308 $[M-OMe]^+$ (16), 151 $[(MeO)_2\ C_6H_3\ CH_2]^+$ (6).

6,7-Dimethoxy 4-(3',4'-dimethoxy benzoyl)isoquinoline (isopapaveraldine) (**7**). 595 mg (1.75 mmol) of isopapaverine were dissolved in a soln of 30 ml HOAc–H_2SO_4 (1:1), then 1.13 g $K_2Cr_2O_7$ were added. After heating at reflux for 1 hr, 100 ml of H_2O were added and the aq. soln was alkalized with NH_4OH, then extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over Na_2SO_4 and evapd under red. pres. The crude product was recrystallized from EtOH; Yield: 65%; mp 174–175°; R_f 0.8; Analysis: $C_{20}H_{19}NO_5$ (353), C,H,N; IR ν^{KBr} cm^{-1}: 1645 (C=O), 1620 (C=N), 1580 (C=C); ^{1}H NMR ($CDCl_3$) δ3, 98 (*s*, 9H, 3 × OMe), 4, 06 (*s*, 3H, OMe), 6, 91 (*dd*, 1H, J = 8 Hz, H-5') 7, 31 (*s*, 1H, H-5), 7, 45 (2*dd*, 1H, J_1 = 8 Hz, J_2 = 2 Hz, H-6'), 7, 6 (*s*, 1H, H-8), 7, 66 (*dd*, 1H, *J* = 2 Hz, H-2'), 8, 52 (*s*, 1H, H-3), 9, 12 (*s*, 1H, H-1); MS, *m/z* (rel. int.): 354 $[M+1]^+$ (24), 353 $[M]^+$ (91), 352 $[M-1]^+$ (8), 338 $[M-Me]^+$ (12), 323 $[M-CH_2O]^+$ (10); 322 $[M-OMe]^+$ (44), 216 $[M-(MeO)_2C_6H_3]^+$ (18), 188 $[M-(MeO)_2C_6H_3CO]^+$ (18), 165 $[(MeO)_2C_6H_3CO]^+$ (100), 77 $[C_6H_5]^+$ (4).

6,7-Dimethoxy 1-hydroxy 4-(3',4'-dimethoxybenzyl) isoquinoline (**9**). In a test tube 65 mg (0.18 mmol) of isopapaverine N-oxide in 15 ml EtOH were exposed with stirring to irradiation from a Philips lamp HPL 125 W for 18 hr. The reaction was followed by TLC. After evapn of the solvent under red. pres. the carbostyril was separated by prep. TLC. (Kieselgel 60F 254 + 366, Me_2CO–CH_2Cl_2, 9:11). The crude product was recrystallized from EtOAc; 25 mg; Yield 38%; mp 246–248°; Rf 0, 73; Analysis: $C_{20}H_{21}NO_5$ (355), C, H, N; IR ν^{KBr} cm^{-1}: 3120 (N–H), 1630 (C=O), 1605 (C=C); ^{1}H NMR 250 MHz ($CDCl_3$) δ: 2.25 (wide *s*, 1H, OH), 3.75 (*s*, 3H, OMe), 3.83 (*s*, 3H, OMe), 3.9 (*s*, 3H, OMe), 4.02 (*s*, 3H, OMe), 4.07 (*s*, 2H, $-CH_2$), 6.69 (*dd*, 1H, *J* = 3, 5 Hz, H-2'), 6, 74 (2*dd*, 1H, J_1 = 8 Hz, J_2 = 3, 5Hz, H-6'), 6.81 (*dd*, 1H, *J* = 8 Hz, H-5'), 7. 07 (*s*, 2H, H-5 and H-8), 7.73 (*s*, 1H, H-3); MS *m/z* (rel. int.): 356 $[M+1]^+$ (27), 355 $[M]^+$ (100), 354 $[M-1]^+$ (5), 340 $[M-Me]^+$ (8), 324 $[M-MeO]^+$ (9), 218 $[M-(MeO)_2C_6H_3]^+$ (7), 151 $[(MeO)_2\ C_6H_3CH_2]^+$ (10).

Acknowledgements—We are grateful to M. T. Bécue who took the mass spectra.

REFERENCES

1. Verdeil, J. L., Bister-Miel, F., Guignard, J. L. and Viel C. (1986) *Planta Med.* 1.
2. Bister-Miel, F., Agier, C., Bury, M., Postaire, E., Guignard, J. L. and Viel, C. (1986) *Plantes Méd. Phytothér.* **20**, 3.
3. Bister-Miel, F., Christinaki, H., Hammoumi, A., Agier, C., Bury, M., Guignard, J. L. and Viel, C. (1986) *Abstracts of the VI International Congress of plant tissue and cell culture* (3–8 August, 1986) p. 202, University of Minnesota, Minneapolis, U.S.A.
4. Bouvier, P., Branceni, D., Prouteau, M., Prudhommeaux, E. and Viel, C. (1976) *Eur. J. Med. Chem.* **11**, 271.
5. Lejay, P., Viel, C. and Uchida-Ernouf, G. (1977) *Il Farmaco, Ed. Sc.* **32**, 3.
6. Lesèche, B., Gilbert, J., Stierlé; A., Stoclet, J. C. and Viel, C. (1978) *Eur. J. Med. Chem.* **13**, 183.
7. Heller, R. (1978) *Physiologie Végétale.* Masson, Paris.
8. Porter, Q. N. (1985) *Mass Spectrometry of Heterocyclic Compounds* (2nd ed.). Wiley, New York.
9. Singer, A. J. and Stern, E. R. (1951) *Anal. Chem.* **23**, 1511.
10. Bister-Miel, F., Guignard, J. L., Bury, M. and Agier, C. (1985) *Bull. Soc Bot. Fr.* **132**, *Lettres bot.* 1, 5.
11. Anonymous *Révélateurs pour la Chromatographie en Couches Minces et sur Papier* p. 13. Merck, Darmstadt.