0.94 ($\underline{CH_3}$ CH NMe), pic large à 1.52 (NH), *m* (4 protons) entre 2.0 et 2.4 (2 CH_2), *s* à 2.41 (NMe), *m* à 2.60 ($\underline{CH}$ NMe), *t* dédoublé ($J = 3.5$, $J' = 7$) à 3.20 ($\underline{CH}$ OMe), *d* à 3.38 (OMe), 6 protons entre 5.5 et 6.4 (CH=); découplage: irradiation de la zone des Me aliphatiques: le *m* à 2.60 devient un *d* ($J = 3.5$), irradiation de la zone des CH_2 vers 2.3: le *t* dédoublé à 3.20 devient un *d* ($J = 3.5$), irradiation du *t* dédoublé à 3.20: le *m* à 2.60 devient un *q* ($J = 7$). Spectre de RMN du ^{13}C: 6 carbones oléfiniques à 136.3, 132.5, 131.5, 130.6, 130.2, 129.5; $\underline{CH}$ (NMe) à 83.8; OMe à 57.9; $\underline{CH}$ (OMe) à 56.3; NMe à 34.2; $\underline{CH_2}$ (CH OMe) à 33.6; $\underline{CH_2}$ Me à 25.9, 2 Me à 14.4 et 13.6. Spectre de masse M^+ 223, pics à *m/e* 191 (M-MeOH), 102, 58. Tartrate: cristallise dans Me_2CO–hexane. F: 119–120°, $[\alpha]_D$: +11° (MeOH $c = 1$); analyse pour $C_{18}H_{31}NO_7$, $\frac{1}{2}H_2O$: calc. %: C 56.54, H 8.37, N 3.66, O 31.41; *tr* %: C 56.53, H 8.15, N 3.48, O 31.76. Chlorhydrate: spectre de RMN ^1H: *t* ($J = 7$) à 1.02 ($\underline{CH_3}$ CH_2), *d* ($J = 7$) à 1.36 ($\underline{CH_3}$ CH NMe), pic large à 2.72 (NMe), *s* à 3.40 (OMe).

Hexahydrodicarprine A **2a**. 0.3 g de dicarprine A en solution dans 40 ml EtOH sont hydrogénés en présence de 0.15 g de charbon palladié à 10% pendant 4 hr à la température ambiante et à la pression atmosphérique. Le catalyseur est séparé par filtration et le filtrat évaporé à sec. Le résidu est chromatographié sur silice. On élue au CH_2Cl_2–MeOH (49:1) 0.18 g de **2a** pur en CCM. Spectre de RMN ^1H: 6 protons entre 0.8 et 1.1 (2 CH_3 aliphatiques), *s* élargi à 1.3 (8 CH_2), *s* à 2.42 (NMe), *m* à 2.77 ($\underline{CH}$ NMe), *m* à 3.20 ($\underline{CH}$ OMe), *s* à 3.38 (OMe); spectre de masse: M^+ 229, pics à *m/e* 171 (M-58), 102 et 58. Chlorhydrate: cristallise dans l'hexane–Me_2CO: F: 95–97°; analyse pour $C_{14}H_{32}ClNO$: Calc %: C 63.24, H 12.13, N 5.27, O 6.02, Cl 13.34; *tr* %: C 62.69, H 12.24, N 5.18, O 6.94, Cl 13.29.

N-Acétylhexahydrodicarprine A **2b**. A une solution de 0.053 g d'hexahydrodicarprine A **2a** dans 0.5 ml MeOH, on ajoute 0.1 ml Ac_2O. Après 30 mn d'agitation à la température ambiante, on dilue à l'eau, alcalinise par de l'ammoniaque et extrait par de l'éther. On obtient 0.058 g de **2b** pur en CCM, mais qui ne cristallise pas; spectre IR: C=O à 1640 cm^{-1}; spectre de RMN ^1H: *s* élargi à 2.1 (COMe), 2*s* (3 protons) à 2.90 et 2.95 (N-Me), 2*s* (3 protons) à 3.37 et 3.41 (OMe) (Le dédoublement des signaux du NMe et du OMe doit être attribué à l'existence de deux conformères); spectre de masse: $M^+ + 1$ 272, pic à *m/e* 100 [Me—CH N(Me)COMe]$^+$.

N-Benzoylhexahydrodicarprine A **2c**. A une solution de 0.086 de hexahydrodicarprine A **2a** dans 3 ml de benzène, on ajoute 1.2 ml de NaOH 0.5 N, puis par petites portions sous agitation magnetique en 15 min 0.5 ml d'une solution de chlorure de benzoyle à 10% dans le benzène. On laisse agiter pendant encore 15 min, puis on dilue à l'eau et extrait par de l'éther. Le résidu est chromatographié sur silice: on élue au CH_2Cl_2-H MeOH (49:1) 93 mg de **2c** pur en CCM, qui ne cristallise pas: DC: λ 275 nm, ε +1.58; spectre IR: C=O à 1640 cm^{-1}, spectre de RMN ^1H: pic large à 2.90 (NMe), pic large à 3.38 (OMe), *s* à 7.30 (C_6H_5); spectre de masse: M^+ 333, pics à *m/e* 331, 162 [Me CH N(Me) COC_6H_5]$^+$.

Dicarprine B **1b**. Spectre UV: λ_{max} 275 nm, ε 50000; spectre de RMN: *t* ($J = 7$) à 1.00 ($\underline{CH_3}$ CH_2), *d* ($J = 7$) à 1.00($\underline{CH_3}$ CH N-Me), pic large à 2.12 (NH et OH), *s* à 2.41 (NMe), *m* à 2.70 (CH NMe), *t* dédoublé ($J = 3.5$, $J' = 7$) à 3.72 ($\underline{CH}$ OH), 6 protons entre 5.3 et 6.4 (CH=); spectre de masse: M^+ 209, pics à *m/e* 191 (M-18) et 58.

Dicarprine C **1c**. Spectre UV: λ_{max} 265 nm, ε: 40000; spectre de RMN ^1H: *t* ($J = 7$) à 1.03 ($\underline{CH_3}$ CH_2), *d* ($J = 7$) à 1.07 ($\underline{CH_3}$-CH NH_2), pic large à 2.42 (NH_2 et OH), *m* à 2.92 ($\underline{CH}$ NH_2), *t* dédoublé ($J = 3.5$, $J' = 7$) à 3.52 ($\underline{CH}$ OH), 6 protons entre 5.3 et 6.6 (CH=); spectre de masse: M^+ 195, pics à *m/e* 177 (M-18) et 44 (Me—$\overset{+}{C}$H=$\overset{+}{N}H_2$).

Remerciements—Nous remercions Madame Allorge pour l'identification de la plante, ainsi que Monsieur P. Potier pour l'intérêt qu'il a porté à ces recherches.

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Phytochemistry, 1978, Vol 17, pp 832–833 Pergamon Press Printed in England

BOUND MORPHINE AND CODEINE IN THE CAPSULE OF *PAPAVER SOMNIFERUM**

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(*Revised received* 17 *October* 1977)

Key Word Index—*Papaver somniferum*; Papaveraceae; poppy capsule; polysaccharide; bound morphine; bound codeine.

Abstract—The polysaccharide fraction of the capsule of *Papaver somniferum* contained bound morphine and codeine. The alkaloids appear to be bound to the polymer by two different types of linkage.

INTRODUCTION

Investigations on *Papaver somniferum* L. have revealed that morphine and other alkaloids are not inert end products of secondary metabolism [1, 2] but active metabolites which are continuously transformed into other compounds [3, 4]. It has also been reported that bound forms of morphine are present in the seeds of *P. somniferum*, perhaps serving a function in seed development [5].

* The present study forms part of the international research programme organized by the United Nations Narcotics Laboratory at the request of the Commission on Narcotic Drugs.

The present work demonstrates that alkaloids are bound also to the H_2O-soluble high MW fraction isolated from poppy capsules freed from seeds.

RESULTS AND DISCUSSION

In the course of studying the H_2O-soluble polysaccharide of the opium poppy capsule it was observed that two Dragendorff-positive compounds were released from the polymer by acid hydrolysis. These compounds had the chromatographic mobility of morphine and codeine. Because of the acidic nature of the poppy capsule polysaccharide [6, 7] it was assumed that alkaloids might form salts with uronic acid residues in the carbohydrate polymer. The existence of such ionic bonds was indicated by the liberation of morphine and codeine under conditions that would normally not affect a covalent linkage. After treatment of the polysaccharide with M NH_4Cl or with M HOAc followed by alkalinification and extraction with $CHCl_3$–*iso*PrOH, morphine and codeine were readily detected by TLC and GLC in the organic fractions M1 and C1 (see Experimental). The residual polysaccharide fraction was subjected to dialysis and then recovered by freeze-drying. Acid hydrolysis of this material resulted in additional release of morphine and codeine, isolated in fractions M2 and C2. The proportion of alkaloids liberated by the two consecutive treatments was (mg/100 g of polysaccharide): morphine: 35 mg in M1; 23 mg in M2; codeine: 21 mg in C1; 10 mg in C2.

A repetition of the first treatment of the polysaccharide (with NH_4Cl or with HOAc) before the acid hydrolysis step gave no further release of alkaloids. This indicates that morphine and codeine are bound to polymer substances in the poppy capsule by at least two different types of bond, one of which appears to be an ionic-type linkage. The other mode of linkage is distinctly more resistant to cleavage, but its nature is unknown at present.

The identification of the alkaloids by GLC and TLC was further confirmed by their UV absorption. In acidic solution morphine and codeine had λ_{max} at 285 nm; in alkaline solution the codeine λ_{max} was unaltered, while that of morphine changed to 298 nm.

Thebaine was not detected with morphine and codeine in the non-dialysable extract. Because of the acid lability of thebaine this alkaloid would hardly survive the acid hydrolysis step. However, even after mild treatment (M NH_4Cl at 40° for 1 hr) no trace of thebaine could be detected. It was demonstrated recently that bound thebaine is present in the capsule, but not in the expelled latex of *P. bracteatum* Lindley [8, 9] and that the alkaloid can be extracted with 5% HOAc. Since the capsule of *P. bracteatum* also contains an acidic polysaccharide (unpublished results) it may be possible that thebaine is bound in a similar way as is suspected for morphine and codeine in the capsule of *P. somniferum*.

The amount of bound alkaloids in the polysaccharide fraction prepared from ripe capsule was much higher than that from unripe capsules. However, it proved difficult to obtain reproducible analytical data for the material of the unripe capsules, possibly due to its tendency to give very viscous and slimy solutions with H_2O.

EXPERIMENTAL

P. somniferum L. var. *album*, of Turkish origin, was grown in the Botanical Garden, University of Oslo, The seeds were kindly provided by Dr O. Braenden, U.N. Narcotics Laboratory, Geneva. A number of capsules were harvested *ca* 2 weeks after petal fall when they were still fresh and green, while the remaining ones were collected after seed-ripening.

Extraction of capsules. Immediately after collection the unripe, green capsules were sliced and immersed in boiling MeOH for 20 min. The MeOH was filtered off and the capsules dried at room temp. The seed-ripe capsules were dry at the time of harvest and were not subjected to this treatment. In the following the two samples of capsules were treated similarly, but only the work with the seed-ripe material is described in detail since only this gave reproducible results. After careful removal of the seeds the residual material was milled to a fine powder (52 g). This was subjected to Soxhlet extraction (8 hr) with petrol, $CHCl_3$ and MeOH respectively; yield after drying, 50.5 g. This product was stirred with H_2O (800 ml) at 50° for 4 hr. Undissolved material was filtered off and the extraction procedure repeated. The combined filtrates were concd to *ca* 400 ml and dialysed against H_2O (3 × 3 l). for 24 hr with stirring. The non-dialysable fraction was freeze-dried; yield, 2.7 g. This product consists essentially of an acidic polysaccharide [6, 7].

Isolation of alkaloids. The freeze-dried extract (0.65 g) was dissolved in M NH_4Cl (30 ml) and kept at 100° for 1 hr. After cooling the pH was adjusted to 12.4 with N NaOH and the soln extracted with 3 × 200 ml of $CHCl_3$–*iso*PrOH (4:1). The combined organic phases were washed with H_2O, dried and evapd to dryness, giving the non-phenolic alkaloid fraction, C1. The aq. phase was acidified with N HCl followed by adjustment to pH 9.2 with 5 M NH_3. The soln was extracted with 3 × 200 ml of $CHCl_3$–*iso*PrOH (3:1) and the combined extracts washed with H_2O, dried and evapd to dryness, giving the phenolic alkaloid fraction M1. The remaining aq. soln was neutralized with HCl, freed from solvent and dialysed against H_2O (2 × 2 l.) with stirring for 16 hr. The retentate was recovered by freeze-drying; yield, 0.54 g. This product was hydrolysed with N HCl at 100° for 2 hr. N NaOH was added to pH 12.4, and the above procedure repeated, this time giving the more firmly bound non-phenolic and phenolic alkaloids, C2 and M2. The fractions C1, C2 and M1, M2, contained codeine and morphine, respectively, and in addition, trace amounts of other unknown compounds as revealed by GLC.

Analytical methods. TLC was carried out on Si gel/UV 254 in solvent systems (1) toluene–Me_2CO–EtOH–25% NH_3 (45:45:7:3) and (2) EtOAc–MeOH–25% NH_3 (17:2:1) both freshly prepared. Compounds were visualized by UV light followed by spraying with Dragendorff reagent. GLC was performed on a chromatograph equipped with FID, using a glass column (60 × 0.2 cm) packed with 3% OV 17 on Gas-chrom Q. Flow rate of N_2: 30 ml/min. For quantitative estimation of morphine and codeine the isolated alkaloid fractions were each dissolved in MeOH (0.30 ml) containing medazepam (7-chloro-2,3-dihydro-1-methyl-5-phenyl-1-H-1,4-benzodiazepine) (1 mg/ml) as int. stand.; the soln was subjected to isothermal GLC at 225°; inj. temp. 250°, detector temp. 280°.

UV measurements of the alkaloid fractions (40–50 μg/ml) were carried out in 0.1N HCl and 0.1N NaOH.

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