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ALKALOIDS FROM CALLUS TISSUE OF *PAPAVER SOMNIFERUM**

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Key Word Index—*Papaver somniferum*; Papaveraceae; alkaloids; callus tissue; norsanguinarine.

Abstract—Norsanguinarine (I), a new alkaloid and 6-acetonyldihydrosanguinarine (II), which appears to be an artefact, have been isolated from callus tissue of opium poppy. Sanguinarine (III), dihydrosanguinarine (IV), oxysanguinarine (V), protopine (VI), cryptopine (VII), magnoflorine (VIII) and choline (IX) have also been detected.

INTRODUCTION

OPIUM poppy, *Papaver somniferum* L., (Keshi in Japanese) is an annual plant and at present cultivated mainly in India, Iran, Turkey, Yugoslavia and Japan. The strain (Ikkanshu) commonly used in Japan yields high quality opium with the highest content of morphine. The presence of alkaloids in callus tissues of opium poppy has been indicated by Ranganathan *et al.*[1] and Staba *et al.*,[2,3] but no alkaloid has been isolated and identified as yet. Rangachari *et al.*[4] and Koblitz *et al.*[5] in reporting phthalic acid ester in callus tissue, made no mention of alkaloid production in these tissues. The present paper deals with the isolation and identification of alkaloids of callus tissues derived from seedling root, stalk and capsule of opium poppy (Keshi-Ikkanshu).

RESULTS

Norsanguinarine (I)

The callus (149 g dry wt) derived from seedling root of opium poppy was extracted with methanol and benzene. The non-phenolic basic fraction obtained from the mixed methanol and benzene extract was chromatographed on silica gel to isolate I.

I gave a pale yellow needles, m.p. 278–280° (decomp.), $C_{19}H_{11}NO_4$ and its UV and IR spectra are clearly compatible with that of the benzophenanthridine alkaloids. The NMR spectrum (CF_3COOH, δ, ppm) revealed two methylenedioxy groups 6·25, 6·50 and two *AB* type protons 7·95, 8·49 and 8·18, 8·52. On the basis of the above spectral data, structure I was assigned to norsanguinarine and finally confirmed by direct m.p. and spectral comparison with a sample recently synthesized as an intermediate of sanguinarine synthesis.[6]

* Part XV in the series "Studies in Plant Tissue Cultures". For Part XIV see T. FURUYA, H. KOJIMA and T. KATSUTA, *Phytochem.* **11**, 1073 (1972).

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[2] D. P. CAREW and E. J. STABA, *Lloydia* **28**, 1 (1965).
[3] E. J. STABA, *Recent Advances in Phytochemistry*, Vol. 2, p. 75, North-Holland, Amsterdam (1969).
[4] P. N. RANGACHARI, B. M. SAYAGAVER and A. F. MASCARENHAS, *Indian J. Chem.* **4**, 191 (1966).
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[6] M. SAINSBURY, S. F. DYKE and B. J. MOON, *J. Chem. Soc.* 1797 (1970).

6-Acetonyldihydrosanguinarine (II)

II, together with I, was isolated by silica gel column chromatography of the mixed methanol and benzene extract. II gave a pale yellow needles, m.p. 194–195·5°, $C_{23}H_{19}NO_5$ and its UV and IR spectra support a benzophenanthridine nucleus having a carbonyl group (1710 cm^{-1}) and are similar to those of 6-acetonyldihydrochelerythrine (X).[7]

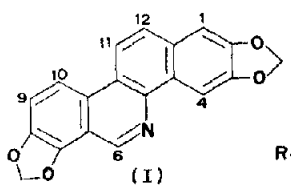
The NMR spectrum ($CDCl_3$, δ, ppm) showed the presence of two methyl groups 2·11 (CH_3CO) and 2·70 (N-CH_3), and two methylenedioxy groups 6·12 and two *AB* type protons 6·94, 7·48 and 7·79, 7·42. The MS exhibited the ion peaks at *m/e* 389 (M^+), 332 (M − 57) and 317 (M − 57 − 15). The occurrence of the ion peak at *m/e* 332 are due to the elimination of acetonyl radical from the side chain at C_6.[8] In addition, the ion peak at *m/e* 317 show the further loss of *N*-methyl radical from (M − 57) ion. From spectral data, structure II was shown to be 6-acetonyldihydrosanguinarine. II was confirmed by direct spectral and m.p. comparison with the synthetic compound, obtained by condensation of sanguinarine with acetone.

Sanguinarine (III), *Dihydrosanguinarine* (IV), *Oxysanguinarine* (V), *Protopine* (VI) *and Cryptopine* (VII)

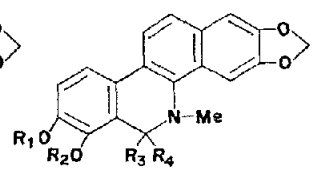
The non-phenolic basic fraction was examined for minor alkaloids by MS and TLC. The presence of III–VII were identified by direct comparison with authentic samples.

Magnoflorine (VIII) *and Choline* (IX)

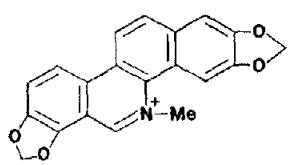
VIII and IX were isolated from the quaternary fraction. VIII was detected by MS and TLC. IX was identified by direct comparison with spectral data and TLC of authentic sample.



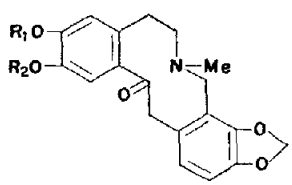
(I)



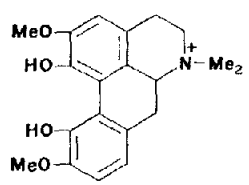
(II) $R_1+R_2=CH_2, R_3=H, R_4=CH_2COMe$
(IV) $R_1+R_2=CH_2, R_3=R_4=H$
(V) $R_1+R_2=CH_2, R_3+R_4=O$
(X) $R_1=R_2=Me, R_3=H, R_4=CH_2COMe$
(XI) $R_1=R_2=Me, R_3=H, R_4=CH_2COCH_2$



(III)



(VI) $R_1+R_2=CH_2$
(VII) $R_1=R_2=Me$



(VIII)

(IX) $Me_3N(OH)CH_2CH_2OH$

DISCUSSION

This is the first time that I has been obtained as a naturally occurring alkaloid. However, it is doubtful whether II is naturally occurring, since it is probably an artefact of the isolation process. Desai *et al.*[7] have reported that X is produced by the action of acetone used

[7] P. D. DESAI, T. R. GOVINDACHARI, K. NAGARAJAN and N. VISWANATHAN, *Indian J. Chem.* **5**, 41 (1967).
[8] J. SLAVÍK, L. DOLEJŠ, V. HANUŠ and A. D. CROSS, *Coll. Czech. Chem. Commun.* **33**, 1619 (1968).

in the isolation, although MacLean *et al.*[9] has mentioned that 1,3-bis(11-hydrochelerythrinyl)-acetone (XI) does not appear to be an artefact produced in the same way. We find that II is present in greater quantity in the acetone extract of opium poppy callus tissue than in the methanol extract and that it is absent from the benzene extract. Therefore, II is an artefact. The alkaloids I–V and VII–IX were detected in the basic fraction of callus tissue derived from capsule and I–V, VIII and IX were also detected in the methanol extract of callus tissues derived from stalk. In fact, the alkaloid pattern on TLC of callus tissues derived from three different organs of opium poppy were almost the same.

In the albino type callus tissues derived in turn from callus tissue of seedling root, stalk and capsule, I was mainly found and the other alkaloids in traces. From this, I appears to be an intermediate in the biosynthesis of sanguinarine type alkaloids in opium poppy callus tissue. Hakim *et al.*[10] have detected the presence of the sanguinarine in opium poppy by paper chromatography and paper electrophoresis, but no benzophenanthridine alkaloid has actually been isolated as yet. Therefore, the isolation of benzophenanthridine alkaloids such as I, II, III and V is for the first time from poppy.

EXPERIMENTAL

M.ps were determined using Büchi melting point apparatus and were not corrected. The NMR spectra were recorded with tetramethylsilane as internal standard. Mass spectra were measured with a JMS-OIS double focusing mass spectrometer with direct inlet system.

Callus culture. Seedling root callus was derived from the seedling of opium poppy, *Papaver somniferum* L. Keshi-Ikkanshu in Japanese), aseptically germinated on White's basal medium in June 1967. The callus tissues from stalk and capsule of opium poppy were derived in June 1966 and June 1967, respectively. Murashige and Skoog's medium (minus glycine) was used for the induction of callus tissues. As plant growth regulators, 2,4-D(2,4-dichlorophenoxyacetic acid) (0·1 mg/l.), IAA(3-indolylacetic acid) (1 mg/l.) and kinetin (0·1 mg/l.), were used for seedling root, stalk and capsule calluses, respectively. They were subcultured every 4 weeks onto fresh modified Murashige and Skoog's medium containing 2,4-D (1 mg/l.), kinetin (0·1 mg/l.) and coconut milk (10%) at 26° in the dark for 3–4 years. The albino type callus tissues, compact and white in color, were selected in culture from seedling root, capsule and stalk callus tissues, respectively. They were subcultured on modified Murashige and Skoog's medium containing 2,4-D (0·1 mg/l.) and kinetin (0·1 mg/l.).

Extraction and isolation of alkaloids. The fresh callus (6·7 kg in fr. wt, 147 g in dry wt) obtained from seedling root were homogenized in cold MeOH by a Waring blender, and were refluxed with MeOH and benzene–MeOH. The combined solution was concentrated under reduced pressure and acidified with HCl. The acidic solution was extracted with light petrol. to remove the neutral and acidic fractions. The aqueous solution was made basic to pH 12 with NaOH and extracted repeatedly with $CHCl_3$-isopropanol (3:1), respectively to yield the non-phenolic basic fraction. The non-phenolic basic fraction was chromatographed over silica gel and eluted with $CHCl_3$–acetone (9:1). Fractions 1–34 (each 50 ml) of the elute were collected. The collected crude fraction was rechromatographed on a column of silica gel with benzene containing increasing proportion of acetone. Fractions 1–5 (each 50 ml) was eluted with benzene and crude IV was obtained, fractions 6–20 (each 50 ml) was eluted with benzene. Evaporation of the solvent gave a pale yellow solid (I). Fractions 37–49 was eluted with benzene–acetone (9:1). The solvent was evaporated to yield crystalline powder (II). Fractions 60–75 (each 50 ml) was eluted with benzene–AcOEt (3:1). Evaporation of the solvent gave III, which was obtained as chloride. Fractions 80–85 (each 50 ml) was eluted with the same system and gave crude V which was purified by preparative TLC. Mixed VI and VII were obtained by preparative TLC. The above alkaline solution was immediately made acidic to Congo red with conc. HCl. Freshly prepared, warm (50–60°), aq. ammonium reineckate solution was added until the alkaloids were completely precipitated. The precipitate was removed by suction filtration. It was dissolved in acetone giving a deep red solution. The solution was treated with hot sat. aq. Ag_2SO_4 until no further precipitation of silver reineckate occurred. The precipitate was filtered off and washed thoroughly with H_2O. The filtrate and washings were combined and aq. $BaCl_2$ was added to convert the quaternary base sulfates into the chlorides and the filtrate from AgCl and $BaSO_4$ was evaporated *in vacuo* to dryness, leaving a slightly yellowish oil. This was dissolved in a small quantity of EtOH, insoluble inorganic substances were removed, and the filtrate

[9] D. B. MacLean, D. E. F. Gracey, J. K. Saunders, R. Rodrigo and R. H. F. Manske, *Can. J. Chem.* **47**, 1951 (1969).

[10] S. A. E. Hakim, V. Mijovic and J. Walker, *Nature, Lond.* **189**, 198 (1961).

was concentrated, leaving a pale yellowish syrupy residue which could not be crystallized. The residue was dissolved in H_2O and cold sat. aq. Na-styphnate added. The styphnate was treated with dil. HCl and KI, and converted into iodide. However, this iodide (VII) was too small to permit further work. The above styphnate filtrate was evaporated *in vacuo* to dryness, leaving yellow residue. This was chromatographed over alumina (Woelm grade III) and eluted with $CHCl_3$–MeOH. The elute was collected, the solvent removed and the residue recrystallized from MeOH–AcOEt to give colorless pillar (VIII).

Norsanguinarine (I). Pale yellow needles (7·8 mg, 0·005%) from acetone, m.p. 278–280° (decomp.) $C_{19}H_{11}NO_4$ (Calcd. 317·068. Obsd. 317·068 by high resolution MS), UV λ_{max}^{EtOH} nm (log ϵ); 215 (4·21) 244 (4·60), 282 (4·50), 296 (4·38), 329 (4·17), λ_{min}^{EtOH} nm (log ϵ); 223 (4·17), 264 (4·33), 322 (4·15), IR ν_{max}^{KBr} cm^{-1}; 1640 (C = N), 1590, 1495, 1450, 1280, 1238, 1182, 1038, NMR (CF_3COOH, δ, ppm), 6·25 and 6·50 (each *s*, 2H, O–CH_2–O in ring *D* and *A*), 7·45 (*s*, 1H, C_1–H), 7·98 (*s*, 1H, C_4–H), 7·95 and 8·49 (each *d*, 1H, J = 10 Hz, C_9–H, C_{10}–H), 8·18 and 8·52 (each *d*, 1H, J = 10, C_{12}–H, C_{11}–H) and 9·55 (*d*, 1H, J = 7·5, C_6–H) and MS, *m/e* 317 (M^+, 100%), 287 (5%), 259 (13%), 229 (6%), 201 (18%). TLC; Silica gel G (Merck) in (*a*) ether-*n*-hexane (1:5), R_f 0·19, and (*b*) benzene–AcOEt (14:1), 0·49. (yellow white fluorescence in UV light).

6-*Acetonyldihydrosanguinarine* (II). Pale yellow needles (3·7 mg, 0·0024%) from MeOH–$CHCl_3$, m.p. 194–195·5°, $C_{23}H_{19}NO_5$ (Calcd. 389·126. Obsd. 389·127 by high resolution MS), UV λ_{max}^{EtOH} nm (log ϵ); 240 (4·43), 288 (4·47), 325 (4·06), λ_{min}^{EtOH} nm (log ϵ); 220 (4·34), 258 (4·06), 313 (3·99), IR ν_{max}^{KBr} cm^{-1}; 1710 (C = O), 1475, 1460, 1440, 1258, 1190, 1042, NMR ($CDCl_3$, δ, ppm), 2·11 (*s*, 3H, CO–CH_3), 2·70 (*s*, 3H, N–CH_3), 6·12 (*s*, 4H, (O–CH_2–O) × 2), 4·95 (*m*, 1H, C_6–H), 6·94 and 7·48 (each *d*, 1H, J = 7·5, C_9–H, C_{10}–H), 7·79 and 7·42 (each *d*, 1H, J = 8, C_{11}–H, C_{12}–H), 7·19 (*s*, 1H, C_1–H), and 7·61 (*s*, 1H, C_4–H) and MS *m/e* 389 (M^+, 35%), 332 (M − 57, 100%) and 317 (M − 57 − 15, 19%). TLC; R_f 0·08 (*a*), 0·39 (*b*). (reddish pink fluorescence in UV light).

Synthesis of 6-*acetonyldihydrosanguinarine* (II). III-Chloride (111 mg) was refluxed with acetone (5 ml) and 20% aq. Na_2CO_3 (1 ml) for 20 hr. The excess acetone was removed under reduced pressure and the residue was dissolved in small amount of H_2O and extracted with Et_2O and chromatographed on a column of silica gel in benzene. The elute was collected, the solvent removed and the residue recrystallized from $CHCl_3$–MeOH to give pale yellow needles, m.p. 194–195·5° (20 mg).

Sanguinarine (III), *Dihydrosanguinarine* (IV), *Oxysanguinarine* (V), *Protopine* (VI) *and Cryptopine* (VII). III-Chloride gave *m/e* 347 (18%), 333 (30%), 332 (M − 35, 10%), 317 (M − 35 − 15, 100%). TLC; (orange fluorescence in UV light). R_f 0·3 (solvent *c*), 0·68 (*d*). IV: GLC; t_R 17·5, TLC; R_f 0·38 (*a*), 0·64 (*b*) (reddish pink). V: *m/e* 347 (M^+, 100%), 319 (M − 28, 8%), R_f 0·33 (*g*), 0·66 (*d*) (blue). VI: GLC; t_R 14·5, TLC; R_f 0·23 (*e*), 0·64 (*f*). VII: GLC; t_R 15·3, TLC; R_f 0·16 (*e*), 0·54 (*f*). Solvent (*c*) benzene–AcOEt (2:1), (*d*) $CHCl_3$–MeOH (20:1), (*e*) cyclohexane–Et_2NH (9:1), (*f*) xylene–MeCOEt–MeOH–Et_2NH (20:20:3:1), (*g*) $CHCl_3$–AcOEt (9:1). GLC condition; JEOL JGC-20K gas chromatograph, packed column with 3% silicone OV-1 on Gas-Chrom Q (100–120 mesh), He flow rate; 2·0 kg/cm^2. Column temp. 235–266° (2°/min); hydrogen flame ionization detector temp. 300°.

Norsanguinarine (I) *in albino-type callus tissue.* The basic fraction of each albino type callus was obtained by the same extraction method as the seedling root callus tissue. TLC; R_f 0·19 (*a*), 0·49 (*b*).

Magnoflorine (VIII). VIII-iodide showed *m/e* 342 (M^+, 22%), 341 (M − 1, 100%), 327 (M − 15, 45%), 325 (M − 17, 17%), TLC; R_f 0·36 (*h*) Silica gel G (Merck) and R_f 0·22 (*i*) alumina (Merck) (blue fluorescence in UV light). Solvent system, (*h*) MeOH–H_2O–NH_4OH (8:1:1), (*i*) $CHCl_3$–MeOH (3:1).

Choline (IX). Colorless rods (100 mg) from MeOH–AcOEt, IR ν_{max}^{KBr} cm^{-1}; 3250 (OH), 2900 (N–CH_3), 1475 (CH_2), 1100 (C = O), NMR (D_2O, δ, ppm), 3·20 (*s*, 9H), 3·51 (*m*, 2H), and 4·05 (*m*, 2H) and MS, *m/e* 121 (M^+, 3%), 89 (M − 32, 35%) and 58 (M − 32 − 31, 100%).

Detection of alkaloids in capsule and stalk callus tissue. The fresh callus (2·9 kg in fr. wt, 65·5 g in dry wt) obtained from capsule was homogenized in cold MeOH by Waring blender and were refluxed with MeOH and MeOH–benzene. The basic fraction was obtained in the same way as above seedling root callus. This basic fraction was prepared for alkaloid detection. The fresh callus (0·6 kg in fr. wt, 17·3 g in dry wt) obtained from stalk was homogenized in cold MeOH by Waring blender and were refluxed with MeOH and MeOH–benzene and this crude extract was prepared for alkaloids detection. The basic fraction and the crude extract were submitted to TLC; silica gel G, alumina (Merck), solvent system (*a*)–(*i*).

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