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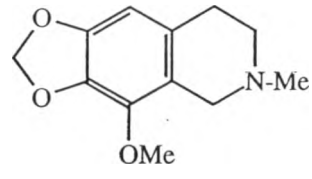
Appendix I

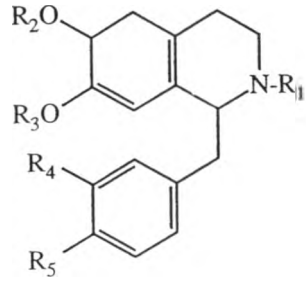
List of alkaliods structure was found in *Papaver somniferum* L.

1. Simple Isoquinoline

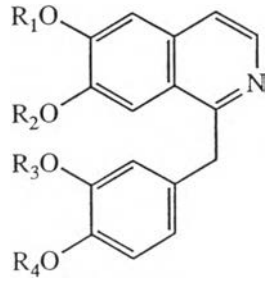


1.1 Hydrocotarnine

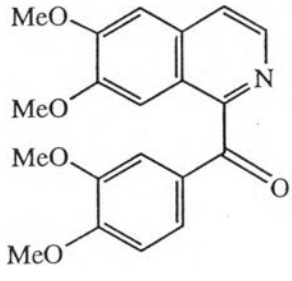
2. Benzylisoquinolines



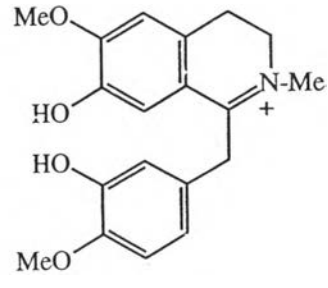
	R_1	R_2	R_3	R_4	R_5
2.1 Codamine	Me	Me	H	OMe	OMe
2.2 Laudanidine	Me	Me	Me	OH	OMe
2.3 Laudanosine	Me	Me	Me	OMe	OMe
2.4 Reticuline	Me	Me	H	OH	OMe
2.5 Tetrahydropapaverine	H	Me	Me	OMe	OMe
2.6 Orientaline	Me	Me	H	Me	H



	R_1	R_2	R_3	R_4
2.7 Papaverine	Me	Me	Me	Me
2.8 Palaudine	Me	Me	H	Me

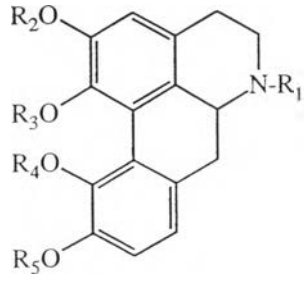


2.9 Papaveraldine

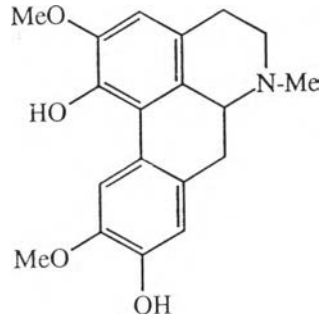


2.10 1,2-Dehydroreticuline

3. <u>Aporphines</u>

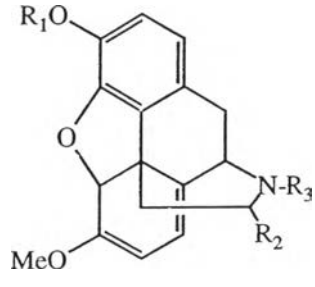


	R_1	R_2	R_3	R_4	R_5
3.1 Corytuberine	Me	Me	H	H	Me
3.2 Magnoflorine	Me2	Me	H	H	Me

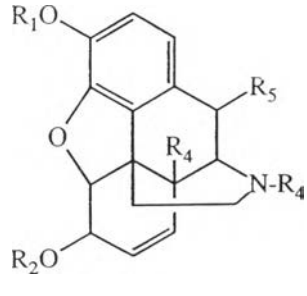


3.3 Isoboldine

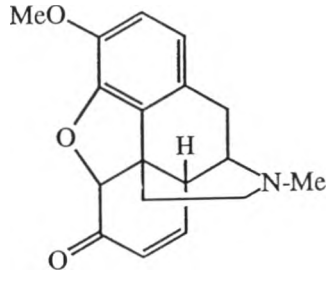
3. Morphinans



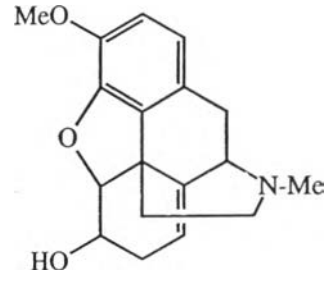
	R_1	R_2	R_3
4.1 Thebaine	Me	H	Me
4.2 Thebaine N-Oxide	Me	H	OMe
4.3 Oripavine	H	H	Me
4.4 16-Hydroxythebaine	Me	OH	Me



	R_1	R_2	R_3	R_4	R_5
4.5 Codeine	Me	H	H	Me	H
4.6 Codeine N-Oxide	Me	H	H	OMe	H
4.7 10-Hydroxycodeine	Me	H	H	Me	OH
4.8 6-Methylcodeine	Me	Me	H	Me	H
4.9 Normorphine	H	H	H	H	H
4.10 Pseudomorphine	H	H	H	Me	H
4.11 Morphine	H	H	H	Me	H
4.12 Morphine N-Oxide	H	H	H	OMe	H

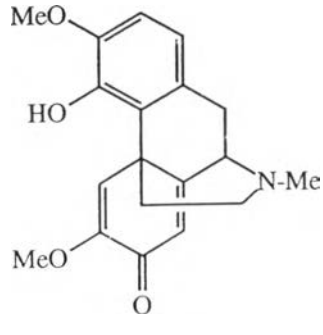


4.13 Codeinone



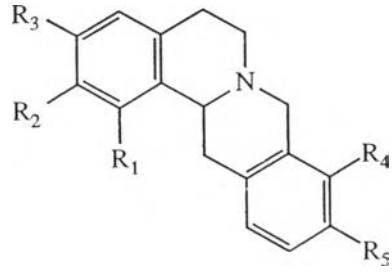
4.14 Neopine

5. Promorphinan

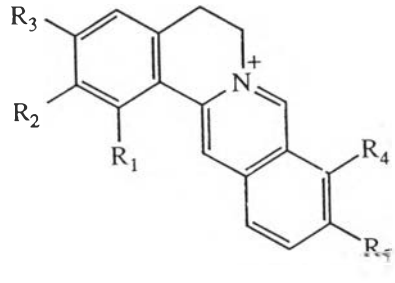


5.1 Salutaridine

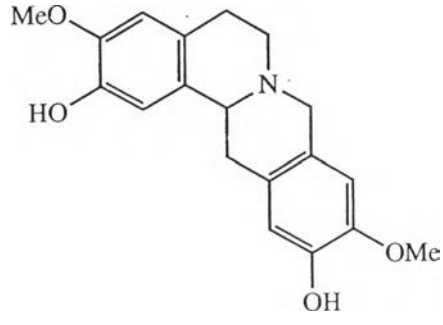
6. Protoberberine



	R_1	R_2	R_3	R_4	R_5
6.1 Canadine	H	$-OCH_2O-$		OMe	OMe
6.2 Scoulerine	H	OH	OMe	OH	OMe
6.3 Isocorypalmine	H	OH	OMe	OMe	OMe
6.4 Stepholidine	H	OH	OMe	OMe	OH

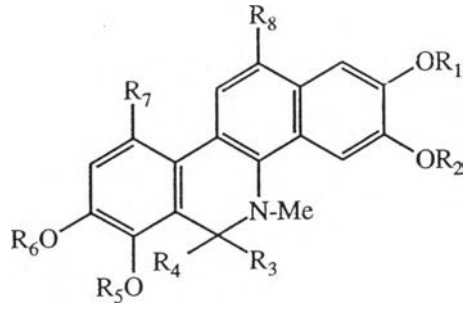


	R_1	R2	R3	R4	R5
6.5 Berberine	H	$-OCH_2O-$		OMe	OMe
6.6 Coptisine	H	$-OCH_2O$		$-OCH_2O-$	

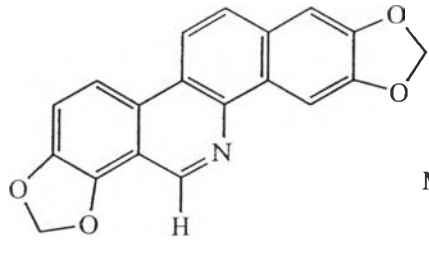


6.7 Coreximine

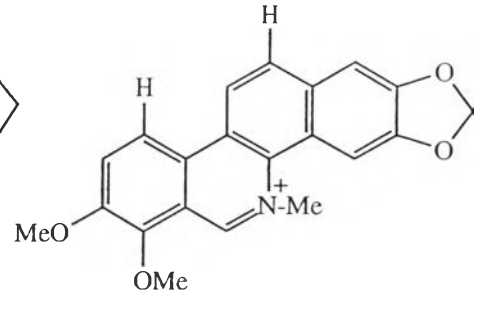
7. Benzophenanthridine



	R_1 R_2	R_3	R_4	R_5 R_6	R_7	R_8
7.1 Dihydrosanguinarine	$-CH_2-$	H	H	$-CH_2-$	H	H
7.2 Oxysanguinarine	$-CH_2-$	-O-		$-CH_2-$	H	H
7.3 6-Acetonyldihydro-sanguinarine	$-CH_2-$	CH_2COCH_3	H	$-CH_2-$	H	H

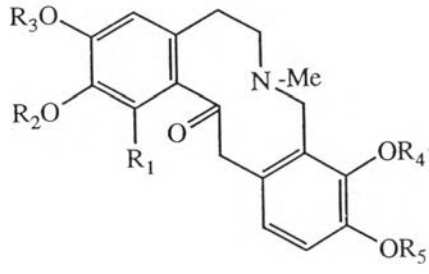


7.4 Norsanguinarine

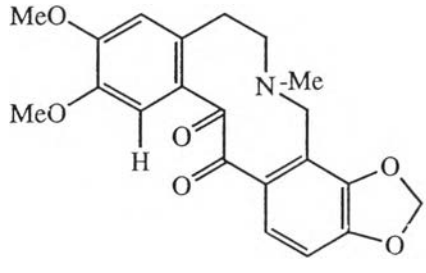


7.5 Sanguinarine

8. Protopines

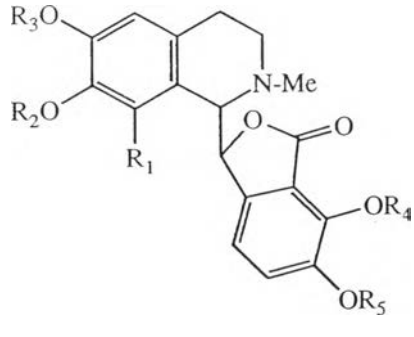


	R_1	R_2	R_3	R_4	R_5
8.1 Protopine	H	-CH_2-		-CH_2-	
8.2 Allocryptopine	H	-CH_2-		Me	Me
8.3 Cryptopine	H	Me	Me	-CH_2-	



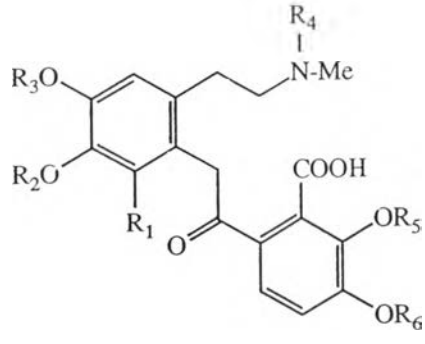
8.5 13-Oxocryptopine

9. Phthalideisoquinolines

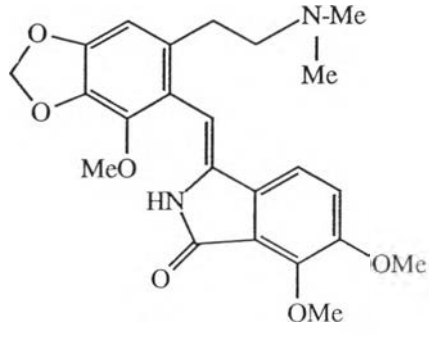


	R_1	R_2	R_3	R_4	R_5
9.1 Narcotine	OMe	-CH_2-		Me	Me
9.2 Narcotoline	OH	-CH_2-		Me	Me
9.3 5'-O-Demethylnarcotine	OMe	-CH_2O		Me	H

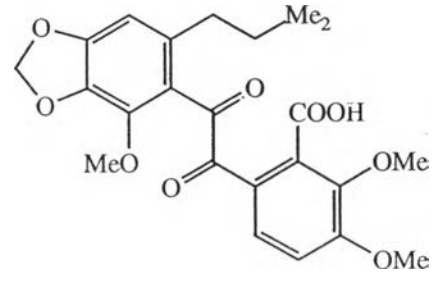
10. Secothalideisoquinolines



	R_1	R_2	R_3	R_4	R_5	R_6
10.1 Nornarceine	OMe	-CH_2-		H	Me	Me
10.2 Narceine	OMe	-CH_2-		Me	Me	Me

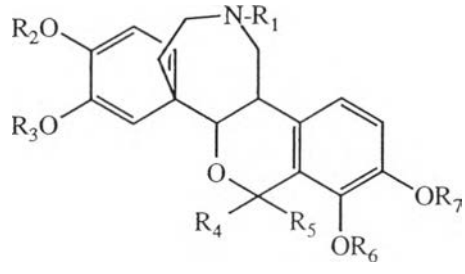


10.3 Narceine imide



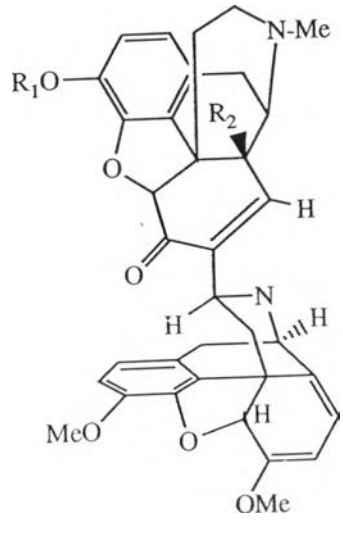
10.4 Narceinone

11. Rhoeadines



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
11.1 Glaudine	Me	Me	Me	H	OMe	-CH_2-	
11.2 Rhoeadine	Me	-CH_2-		H	OMe	-CH_2-	
11.3 Papaverrubine C	H	Me	H	OMe	H	-CH_2-	
D	H	Me	H	H	OMe	-CH_2-	

12. Dimeric Isoquinolines



	R_1	R_2
12.1 Somniferine	H	OH
12.2 Somniferine O-methyl ether	Me	OH

APPENDIX II

Table 9 **Ammonium Sulfate Precipitation Table** (Harris and Angal, 1989)

The amount of solid of ammonium sulfate to be added to a solution to give the desired final saturation at 0°C

	Final concentration of ammonium sulfate, %saturation at 0°C																
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Initial concentration of ammonium sulfate																	
	g solid ammonium sulfate to add to 100ml of solution																
0	10.7	13.6	16.6	19.7	22.9	26.2	29.5	33.1	36.6	40.4	44.2	48.3	52.3	56.7	61.1	65.9	70.7
5	8.0	10.9	13.9	16.8	20.0	23.2	26.6	30.0	33.6	37.3	41.1	45.0	49.1	53.3	57.8	62.4	67.1
10	5.4	8.2	11.1	14.1	17.1	20.3	23.6	27.0	30.5	34.2	37.9	41.8	45.8	50.0	54.5	58.9	63.6
15	2.6	5.5	8.3	11.3	14.3	17.4	20.7	24.0	27.5	31.0	34.8	38.6	42.6	46.6	51.0	55.5	60.0
20	0	2.7	5.6	8.4	11.5	14.5	17.7	21.0	24.4	28.0	31.6	35.4	39.2	43.3	47.6	51.9	56.5
25		0	2.7	5.7	8.5	11.7	14.8	18.2	21.4	24.8	28.4	32.1	36.0	40.1	44.2	48.5	52.9
30			0	2.8	5.7	8.7	11.9	15.0	18.4	21.7	25.3	28.9	32.8	36.7	40.8	45.1	49.5
35				0	2.8	5.8	8.8	12.0	15.3	18.7	22.1	25.8	29.5	33.4	37.4	41.6	45.9
40					0	2.9	5.9	9.0	12.2	15.5	19.0	22.5	26.2	30.0	34.0	38.1	42.4
45						0	2.9	6.0	9.1	12.5	15.8	19.3	22.9	26.7	30.6	34.7	38.8
50							0	3.0	6.1	9.3	12.7	16.1	19.7	23.3	27.2	31.2	35.3
55								0	3.0	6.2	9.4	12.9	16.3	20.0	23.8	27.7	31.7
60									0	3.1	6.3	9.6	13.1	16.6	20.4	24.2	28.3
65										0	3.1	6.4	9.8	13.4	17.0	20.8	24.7
70											0	3.2	6.6	10.0	13.6	17.3	21.2
75												0	3.2	6.7	10.2	13.9	17.6
80													0	3.3	6.8	10.4	14.1
85														0	3.4	6.9	10.6
90															0	3.4	7.1
95																0	3.5
100																	0

Table 10 Solutions for SDS-polyacrylamide gel electrophoresis (Laemmli, 1970)

Solution	Composition	Procedures
Sample buffer	Distilled water 4.0 ml 0.5M Tris-HCl pH 6.8 1.0 ml Glycerol 0.80 ml 10%w/v SDS 1.60 ml β-mercaptoethanol 0.40 ml 0.05%w/vBromophenol blue 0.2ml.	Dilute the sample at least 1:4 with sample buffer, and heat at 95°C for 4 min.
Running buffer	Tris base 9 g Glycine 43.2 g SDS 3 g to 600 ml with H_2O	Dilute 60 ml 5xstock with 240 ml H_2O for one electrophoresis run. Store at 4°C. Warm to 37°C before use if precipitation occurs.
Lower gel buffer	1.5M Tris-HCl,pH 8.8: 27.23 g Tris base	Adjust to pH 8.8 with 1N HCl. Make to 100ml with distilled water and store at 4 °C
Upper gel buffer	0.5M Tris-HCl, pH 6.8: 6 g Tris base	Adjust to pH 6.8 with 1N HCl. Make to 100ml with distilled water and store at 4°C
Acrylamide stock	Acrylamide/Bis(30%T,2.67%C): 87.6 g acrylamide 2.4gN',N'-bismethyleneacrylamide	Make to 300ml with distilled water. Filter and store at 4°C in the dark(30 days maximum). Acrylamide is a neurotoxin; do not breathe dust or allow to touch skin. Do not mouth pipette.
10%Ammonium persulfate	100 mg ammonium persulfate	To make the 10%ammonium persulfate slution, dissolve 100mg APS in 1 ml H_2O. Freshly prepared daily, store at 4°C
N,N,N',N'-Tetramethylenediamine		Store at 4°C
10%SDS	10 g SDS to 100 ml with distilled water	Dissolve 10 g SDS in water with gentle stirring and bring in 100 ml with H_2O

Table 11 SDS-polyacrylamide gel electrophoresis (linear slab gel)

Step of procedures	Procedures
1.Preparing the gel	Assemble gel sandwich according to the manufacturer's instructions in the case of commercial apparatus(eg. Bio-Rad Mini-Gel). Prepare the separating gel monomer solution and pour the solution smoothly using an automatic pipet. Immediately overlay the monomer solution with water. Allow the gel to polymerize for 45 min. to 1 hr, rinse off the overlay solution. Prepare stacking gel monomer solution. Carefully insert comb into gel sandwich until bottom of teeth reach top of front plate. Pipet the stacking gel solution onto separating gel until solution reaches top of front plate. Allow the gel to polymerize for 30-45 min. After stacking gel has polumerized, remove comb carefully. Place gel into electrophoresis chamber. Add electrophoresis buffer to inner and outer reservoir, making sure that both top and bottom of gel immersed in buffer.
2.Preparing and Loading sample	Combine protein sample and sample buffer in an Eppendorf tube. Heat at 100^{o}C for 2-10 min. Spin down protein solution for 1 sec. Introduce sample solution into well using Elec ^{TM} Tip
3.Running a gel	Attach electrode plugs to proper electrodes. Current should flow towards the anode. Turn on power supply to 200V. When dye front migrate to the bottom of the gel in 40 min., turn off the power supply. Remove electrode plugs from electrodes. Remove the gel plates from electrode assembly. Carefully remove a spacer, gently pry apart the gel plates. Later, the gels are to be stained.

Table 12 Polyacrylamide gel staining procedure using Silver Stain Plus^{R} (Merril *et al.*, 1984)

The following preparations are adequate for staining two minigels(8x10x0.75mm)
For silver staning, approximately 0.1 ug of protein per band is needed for visualization.

Step of Procedures	Procedures
1.Fixative Step-20min	Fixative Enhancer Solution Preparation Methanol 50%V/V Acetic Acid 10%V/V Fixative Enhancer Concentrate 10%V/V Deionized Distilled Water 30%V/V After gel electrophoresis, place gels in the Fixative Enhancer Solution. With gentle agitation fix the gel for 20 minutes.
2.Rinse Step-20 min	Rinse the gels in 400 ml deionized deistilled water for 10 minutes with gentle agitation. After 10 minutes, place an additional water for 10 minutes.
3.Staining and Developing Step-20 min	Place 35 deionized water into a large beaker and stir with a Teflon coated stirred bar. Add the following to the beaker in this order 5.0 ml Silver Complex Solution 5.0 ml Reduction Moderator Solution 5.0 ml Image Development Reagent Immediately before use quickly add 50 ml of the room temperature Development Accelerator Solution to the beaker. Swirl well. Add the contents of the beaker to the staining vessel. Stain with gentle agitation. Stain the gels for approximately 20 minutes or until desired staining intensity is reached.It may take at least 15 minutes before the bands first become visible. Note:Staining time is dependent on the sample and the quantity loaded. After the desired staining is reached, place the gels in 5% acetic acid to stop the reaction.
4.Stop Step-15 min	Prepare a 5% acetic acid solution to stop the staining reaction. Place gels in stop solution for a minimum of 15 minutes. After stopping the reaction rinse the gels in high purity water for 5 minutes. The gels are then ready to be dried or photographed.

Table 13 Coomassie blue staining

Approximately 1 μg of protein per band is needed for detection when gels are stained with Coomassie blue

Steps	Reagent	Procedures
1.Staining	Coomassie blue staining solution Methanol 40% Acetic acid 10% Coomassie blue R-250 0.25% Distilled water	Remove the gel from the gel sandwich. Lift the glass plate to remove the gel. Place gels in staining solution for 1/2 to 1 hour.
2.Destaining	Coomassie blue destaining solution Methanol 40% Acetic acid 10% Distilled water	After staining, destain the gel in destaining solution. If a gel destainer is not available, immerse the gel in destaining solution and place it on a shaking platform. Change the destain solution as often as is necessary until the background is clear, usually 3-4 times. Destaining usually takes 2-3 hours.

VITA

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