PART I

INTRODUCTION

Datura metel L. var. fastuosa Safford (Smitinand 1980) is an annual herbaceous plant belonging to the family Solanaceae, orNightshade family and is distributed primarily in tropical and South America (Benson 1959 ; Lawrence 1970). All parts of this plant contain chiefly tropane alkaloids, hyoscine (also known asscopolamine) and in lesser amount l-hyoscyamine. The latter racemizes during the drying and extraction processes to dl-hyoscyamine or atropine (Punyarajun 1982). Both hyoscine and atropine are used worldwide in medicine as mydriatic and parasympatholytic agents (Innes and Nickerson 1980 ; Shah and Khanna 1964; Somanabandhu and Suntorncharoenon 1980 ; Tyler et al. 1981). In Thailand, Datura species are used in traditional medicine more than 4.5 tons annually and are distributed widely in the wastelands and the road-sides (Punyarajun and Tipduangta 1981). It thus serves as a commercial source of the medicinally important tropane alkaloids, namely atropine and hyoscine. Because of its widespread occurrence throughout Thailand, it may be considered as an attractive source for the production of medicinally useful tropane alkaloids, particularly hyoscine. This will replace the imported atropine sulfate, Fluidextract Belladonna, Fluidextract Hyoscyamus, Fluidextract Stramonium, mydriatic eyedrops and other solanaceous plants which contain similar alkaloids, so as to reduce the cost of drug importation spent each year. Although the domestic market is small in this respect it is one of the medicinal plants suggested by the UNIDO to grow in developing countries (Bunyaprapattara 1980-1981) so that they can be self reliance for the pharmaceuticals which are used as the GI-antispasmodics and more demand is expected in the future. Therefore, optimal cultivated conditions as well as optimal time of harvesting should be established for the commercial exploitation of this medicinal plant.

1) Botanical Characteristics of the Family Solanaceae

The family Solanaceae is found in the tropics but well represented also in temperate region (Bailey et al. 1975 ; Hutchinson 1973; Rost et al. 1984). There are about 85-90 genera and over 2,000 species (Bailey et al. 1975 ; Hawkes et al. 1979 ; Hutchinson 1973), yielding many subjects for ornament as also for food and drugs. Although the family is of worldwide distribution, most of the cultivated form were brought under domestication in the Western Hemisphere. There are many poisonous and medicinal plants in this family (Hutchinson 1973; Rost et al. 1984).

It is mostly glandular plant whose herbage is variously odoriferous when crushed (Lawrence 1955). It is a coarse, erect or climbing herbs, shrubs or small trees (Bailey et al. 1975) with bicollateral strands (Benson 1959 ; Porter 1967). The leaves are mostly alternate entire or variously dissected or pinnate (Benson 1959 ; Bailey et al. 1975 ; Lawrence 1970). The inflorescence is cymose and the flowers are mostly bisexual, regular, hypogynous discs, gamopetalous corolla prevailingly rotate but varying greatly in its shape and structure (Bailey et al. 1975 ; Lawrence 1955, 1970 ; Porter 1967). Sepals are usually coalescent for almost their full length (Lawrence 1970). Calyx is 5 lobed, persistent and enlarging in fruit (Bailey et al. 1975 ; Benson 1959 ; Lawrence 1970). Corollas are in rotate to tubular form, typically 5-lobed with usually plicate or convolute (Benson 1959; Lawrence 1970). The androecium is commonly of four didynamous stamens (a fifth is usually represented by staminode) (Benson 1959 ; Lawrence 1955). Stamens are epipetalous, alternating with corolla lobes, often connivent by their anthers and hypogynous disc usually present. The gynoecium consists of a pistil with a superior usually bilocular ovary having axile placentation and a single style with two-lobed stigma (sometimes 3-5 loculed by false septation). The fruits are berries or septicidal capsules with smooth or pitted seeds (Benson 1959 ; Lawrence 1955, 1970 ; Porter 1967).

The family Solanaceae was interpreted by Wettstein as probably of a polyphyletic origin, as evidenced by alliance with several families. The most related family is the Scrophulariaceae and can be distinguished from the Solanaceae by the presence of the actinomorphic corolla, the typical 4 or 5 stamens, the usually plicate corolla and the invariable anatomical character of bicollateral vascular strands in the latter (Benson 1959 ; Hawkes et al. 1979 ; Lawrence 1970). The family was divided by Wettstein into 5 tribes : Nicandreae, Solaneae, Datureae, Cestreae and Salpiglossideae (Hawkes et al. 1979 ; Lawrence 1970).

This family was included by Bentham and Hooker and by Bessey within the Polemoniales (separated by Bessey from his Scrophulariales by the actinomorphic corolla), whereas Halliers considered it to be the primitive member of the Tubiflorae and (together with the Scrophulariaceae) to have been derived probably from the Linaceae (Benson 1959 ; Hawkes 1979 ; Lawrence 1970). Hutchinson included it as the primitive taxon of his Solanales, together with the Convolvulaceae, an order ancestral to his Personales (Lawrence 1970).

2) Botanical Characteristics of the Genus Datura.

The genus Datura belongs to the tribe Datureae and the family Solanaceae. The generic name of Datura was first used by Linnaeus (1737) in his Hortus Cliffortianus (Satina et al. 1959). According to Safford (1922), Linnaeus latinized the East Indian name Dhatura or Dutra. According to Asa Gray (1848), the name Datura is from the arabic name Tatorah (Satina et al. 1959). More than a score of common names in English and many more in other languages are used for this cosmopolitan weed of worldwide distribution (Kingsburg 1964).

This genus comprises of 25 species (Pongpisal, unpublished M.Sc. thesis). Of these, several are found in warmer part of the globe and several others are occasionally cultivated for their great trumpet-like odorous flowers but some are widespread weeds (Bailey et al. 1975 ; Kingsburg 1964). Blakeslee (Satina et al. 1959) gave a simple description of Datura as follows :

Flowers perfect, gamopetalous, solitary and pediceled, rather small in some species and very large in others. Calyx elongated and tubular, evenly or unevenly five-lobed or spathelike, splitting lengthwise, or circumscissile with the base and breaking away after flowering or forming an enlarged and subtended disk, or cup, under the capsule. Corolla funnel-shaped and tubular. The tube long and slender, the limb either five-lobed or forming a ten-toothed and plaited border. The five stamens, alternating with the corolla lobes, are attached to the inner surface of the tube near the base. Stigma two-lobed, style filiform. Ovary superior, two celled at the top and often four celled below because of the presence of a false septum. Fruit a capsule, globose, fusiform, or ovoid, four-valved or bursting irregularly, spiny or smooth. Leaves and stem glabrous or pubescent. Annual or occasionally perennial herbs, bushes or small trees.

The genus Datura is divided by Safford (1921) into four sections : I. Stramonium II. Dutra III. Ceratocaulis IV. Brugmansia (Satina et al. 1959 ; Hawkes et al. 1979).

2.1 Section I. Stramonium [Tournefort] Bernhardi. (Satina et al. 1959).

The species belonging to this section, D. stramonium, D. ferox and D. quercifolia have erect flowers and capsules which when ripen, break regularly into four valves. The calyx is evenly five-toothed, circumscissile. The seeds are black. Each fork of the dichotomous stem has a flower bud. These species are erect and annual herbs.

2.1.1) D. Stramonium L. (D. tatula L., D. inermis Jacq.) (Satina et al. 1959 ; Bailey et al. 1975 ; Benley and Trimen 1983; Kingsburg 1964 ; Trease and Evan 1985).

D. stramonium, D. tatula and D. inermis have been considered as varieties by many botanists. Its stem and branches are round, green and nearly glabrous. The leaves of this plant have petioles about one-quarter to one-third of the length of lamina. The lamina is ovate and coarsely dentately lobed with a few irregular dentations on the lobe. The corolla is tubular-funnel shaped. It is white or violet. The calyx is half of the corolla tube and dropping with the corolla shortly after the withering of the latter. The anther is purple in the purple - flowered types and white in the white types. The style is shorter than the corolla tube. The mature capsule is almost completely 4 celled. The seeds are numerous and having kidney shaped.

2.1.2) D. ferox L. (Stramonium ferox Boccone.) (Satina et al. 1959).

This species can be easily recognized by the large and very stout spines covering the capsules. Upper spines are larger than the lower. The stem is green except the hypocotyl which is purple. The leaves are angular and sinuate. The corolla and the anthers are white. The capsule are ovoid. Other characters are as the same as D. stramonium.

2.1.3) D. quercifolia H.B.K. (Satina et al. 1959).

It is a coarse, branched herb or short-lived shrub. The stem and leaves are slightly downy and pubescent. The leaves are deeply pinnately lobed. The anthers and the stem are purple. The capsule and its spines are as the same as in D. ferox but less stout than the latter.

2.2) Section II. Dutra Bernhardi. (Satina et al. 1959).

Six species belong to this section : D. pruinosa, D. leichhardtii, D. meteloides, D. metel, D. discolor and D. innoxia. The flowers are erect, small or very large, five lobes or ten - angled, white or colored, with simple or double corolla. The calyx is usually dropping with the withered corolla and circumscissile. The capsules are nodding or inclined, breaking irregularly at maturity, spiny or tuberculate. The seeds are brown or black with strophiole attached to the hilum. These species are annual herbs, occasionally forming perennial roots.

2.2.1) D. pruinosa Greenm. (Satina et al. 1959).

It is a slightly spreading stem. The leaves are dark green, ovate and slightly dentate with unequal at the base. The corolla is white. The calyx, leaves and stems are densely pubescent. The capsule is nearly globular and covered with short slender spines.

2.2.2) *D. leichhardtii* Muell. (Satina *et al*. 1959).

This species, like the preceding, differs from other species in the *Dutra* section by the smaller size of its flowers, leaves, capsules and general habits.

2.2.3) *D. meteloides* DC. in Dunal. (Satina *et al*.1959).

The corolla is white and faintly suffused with violet. The stigma is frequently protrudes from the corolla in buds. The anthers are white. Other characters are as the same as *D. pruinosa.*

2.2.4) *D. metel* L. (*D. fastuosa* L., *D. alba* Nees.) (Satina *et al*. 1959 ; Sobti and Kaul 1982 ; Kingsburg 1964 ; Perry *et al*. 1980 ; Quisumbing 1951 ; Shan and Khanna 1965).

So much work has been done on *Datura metel* that is difficult to draw up a summary. The two names given as synonyms are regarded as two races of the species by those who have worked cytologically with it. This is a glabrous spreading herb sometimes becomes shrubby or erect. It is about 0.3 meter in some varieties, reaching to 1.5 meters in others. The plant branches dichotomously, the older plants bearing leaves at the top only. The main stem is moderately thickened in the perennial, but not in the first year. Stems are green in forms with white flowers and purple in those with purple flowers. The young stems are fragile and glabrous. The distal parts of stem have dwarf shoots of 3-4 smaller leaves, which arise in the axils of the leaves, off the main branches. The leaf scars are conspicuous on the stems. The leaves are olive green above and pale green below. The larger leaves on the main branches have an alternate phyllotaxis. Petiole is about 5 - 8 cm. long, flat above, and glabrous. Lamina is about 15 - 35 cm. long and 8 - 12 cm. broad, ovate to oblong ovate with unequal base, nearly entire or with a few teeth, minutely pubescent on both the surfaces. Venation is reticulate with 3 - 5 principal lateral veins on each side, which anastomose at the margin. Apex is acute. Base is asymmetric. The smaller leaves of the dwarf shoots are in close group of 2 - 5 out of which generally 2 are bigger. The flowers are large and white. The corolla is trumpet-shaped, simple, double, triple by the irregular petaloid outgrowth of the stamens and inner corolla surfaces ; about 14 to 15 cm. long. The calyx is regular, 5 to 7 cm. long, evenly five lobed, less than half as long as the corolla. Style is 11 to 13 cm. long. Capsule is globose, inclined, 4 to 6 cm. in diameter and covered with very short spines and tubercles.

The main characters that distinguish this species from the other large species of the _Dutra_ section are the glabrous condition of the stem and leaves and the very short spines or tubercles on the capsule.

2.2.5) _D._ _innoxia_ Mill. (Satina _et_ _al_. 1959 ; Sobti and Kaul 1982)

It is closely resembles _D._ _metel_ and is distinguished by the presence of dense pubescense, ovate leaves with cordate base and long weak spines on the fruit which is capsule with locular dehiscence.

2.2.6) D. discolor Bernh. (Satina et al. 1959)

The habit is spreading. The leaves are ovate, dentate or almost entire. The corolla is nearly white with violet striped throat. The calyx is sharp angled, dropping with corolla and leaving at the base of the capsule a large frill. The capsule is ovoid and covered with long slender spines. The stem and leaves are slightly pubescent.

2.3) Section III. Ceratocaulis (Spach.) Bernhardi. (Satina et al. 1959).

2.3.1) D. Ceratocaulis Ort. (Satina et al. 1959).

It is the single species of this section and differ from other herbaceous forms of Dutra by having smooth, berrylike capsules. The flowers are large, pale lavender (darker along the vein) and trumpet-shaped. The corolla tube is long, narrow and ten-toothed. The calyx is spathelike. The stem is smooth. The leaves are pinnately lobed and slender beneath. Seeds are black, smooth and shiny.

2.4) Section IV. Brugmansia (Persoon) Bernhardi. (Satina et al. 1959).

The taxonomy of the section Brugmansia is rather complicated. They are widely cultivated as ornamentals. They produce large, white or coloured trumpet-shaped flowers. The calyx is spathelike or toothed, not circumscissile at the base and falling off entirely or persisting as a husk about the base of the fruit. The fruit is unarmed, spheroid, oblong, lemon shaped, or long and slender, opening irregularly. The seeds are large and covered by a thick corky layer. The stems are woody ; shrub or small trees of tropical America, especially Colombia, Ecuador, Peru and Brazil.

3. Botanical Characteristics of *Datura Metel* var. *Fastuosa*
(Satina *et al*. 1959 ; Shah and Khanna 1965).

Botanical name : *Datura metel* Linn. var. *fastuosa* Safford (Smitinand 1980)

Family : Solanaceae (Satina *et al*. 1959 ; Hawkes *et al*. 1979)

Vernacular name : Lamphong kaasalak (Central, Sukhothai) ; Ma khuea baa dok dam (Lampang) ; kaasalak. (Smitinand 1980)

D. metel L. var. *fastuosa* Safford and *D. metel* Linn. are known as "black" and "white" datura respectively (Punyarajun and Tipduangta 1981 ; Shan and Khanna 1963, 1965). For a long time confusion prevailed about the correct botanical identities of *D. metel* var. *fastuosa*. Shah and Khanna (1965) elucidated and pointed out the botanical identities of *D. metel* var. *fastuosa* which can be distinguished from D. *metel* by the purple colour of the stem, main veins, flowers and double or triple corolla.

D. metel var. *fastuosa* is an annual herb, but becomes a perennial undershrub. The plant branches dichotomously, the older plants bearing leaves at the top only. The main stem branches at higher region than *D. metel* and is not spreading as is the case with *D. metel.* Colour of the stem, petiole and lower surface of the young leaves is deep purple. Corollas of the multiple flowers are also purple. The plant attains a higher altitude and becomes a shrub or a small tree sometimes. It attains a height up to 10 ft. The lower thick stem with cork developed is ash coloured. The petiole and the veins on the lower side are purple in colour, sometimes patches of lamina are also purple colour on the lower side, especially in young leaves. The most characteristic feature of the plant is the flower, which has double, triple or even quadruple corolla, purple coloured and veined. In case of *D. metel*, the flowers are mostly creamy white to white, usually having a single corolla. Other characters are as same as in *D. metel.*

4. Chemistry of Tropane Alkaloids in the Genus *Datura*.

Safford (1921) grouped the genus *Datura* into four sections: I. *Stramonium* ; II. *Dutra* ; III. *Ceratocaulis* and IV. *Brugmansia* (Hawkes *et al.* 1979 ; Leete 1959). In recent years a number of reports have appeared expressing wide variations in the total alkaloid content. Table 1. (p.13) (Leete 1959) lists the various species of *Datura* and their alkaloid content as far as they are known. The percentage weight of the alkaloids are average value, since different workers have reported varying yields of alkaloid.

Table 1. Alkaloid Contents of <u>Datura</u> Species*

Species	Alkaloids	References
<u>D. stramonium</u>	Hyoscyamine 0.4 (in leaves) 0.2 (in stems) 0.1 (in roots) Hyoscine 0.01 (in leaves) 0.05 (in stems) 0.1 (in roots) 0.2 (in very young plants) 3,6-ditiglylteloidine 0.01 (in roots) Cuscohygrine (in roots)	Andrews (1911) Braun (1939) Evans and Partridge (1957) Feldhaus (1905) Guillon (1950) van Haga (1954) Hegnauer (1953) Hemberg and Fluck (1953) Jackson and Rowson (1953) Johnson and Nuner-Melendez (1942) Otsuka and Nagata (1953) Rowson (1945) Schratz and Spaning (1942) Sirgo (1939) Steinegger (1951, 1953a) Beal et al. (1954) Evans and Menendez (1956) Evans and Partridge (1953a, 1957) Jentzsch (1953) Rudorf and Schwarze (1951) Shilbata and Imaseki (1954) Shibata et al. (1951) Steinegger (1953a, b) Jentzsch (1953) Kurowicka-Kuleszyna (1953) van Os et al. (1955)
<u>D. stramonium</u> "tatula"	Hyoscyamine 0.15 Hyoscine 0.07 3,6-ditiglylteloidine 0.01 (in roots)	
<u>D. stramonium</u> "inermis"	Hyoscine 0.1 (before flowering) 0.2 (after flowering) Hyoscyamine 0.04 (before flowering) 0.07 (after flowering)	
<u>D. ferox</u>	Hyoscine 0.3 Meteloidine 0.1 (none in seeds) 3,6-ditiglylteloidine 0.05 (in roots) Cuscohygrine (in roots) An unknown alkaloid $C_{15}H_{20-22}O_{4}N$	Evans and Partridge (1949; 1953a, b; 1957) van Haga (1954) Romeike and Zimmermann (1958)

Table 1 (continued)

Species	Alkaloids	References
<u>D. quercifolia</u>	Hyoscyamine 0.4 (in leaves) 0.3 (in seeds)	Kirchner (1985) Stary (1952)
<u>D. metel</u>	Hyoscine 0.1 Hyoscyamine 0.04 (disappears after flowering) Nor-hyoscyamine 0.01 Cuscohygrine (in roots)	Andrews (1911) Carr and Reynolds (1912) Guha (1951) van Haga (1954) Jentzsch (1953) Prasad (1948) Shibata and Imaseki (1954) Pradisth and Santos (1939a,b)
<u>D. metel</u> "alba"	Hyoscine (main alkaloid) Hyoscyamine (a trace)	Andrews (1911)
<u>D. metel</u> "fastuosa"	Hyoscine 0.1 Hyoscyamine 0.02 (none in seeds or roots)	
<u>D. meteloides</u>	Hyoscine 0.1 Meteloidine 0.05 Hyoscyamine 0.03 Nor-hyoscyamine 0.02	Carr and Reynolds (1912) Pyman and Reynolds (1908) Shibata and Imaseki (1954)
<u>D. innoxia</u>	Hyoscine 0.3 (total plant) 0.04 (in seeds) Hyoscyamine (with meteloidine) 0.06 (total plant) 0.1 (in seeds) Cuscohygrine (in roots) An unknown alkaloid, m.p. 44-6° C	Evans and Partridge (1953a,b,c) Fluck and Nisoli (1954) Gerlach (1948) van Haga (1954) James (1953) Steinegger and Gessler (1955)
BRUGMANSIA "<u>D. arborea</u>"	Hyoscine 0.4 Hyoscyamine (in young stems and roots)	Kirchner (1905) Schmidt (1906) Rolando-Suarez (1952)

Table 1 (continued)

Species	Alkaloids	References
"*D. sanguinea*"	Hyoscine 0.35 (in aerial parts) 0.2 (in roots) Hyoscyamine 0.02 (in aerial parts) 0.4 (in roots) Several other alkaloids not characterized with certainty	Dray and Foster (1953)
"*D. suaveolens*"	Hyoscyamine 0.1	Simoes (1951)

*The alkaloids were isolated from the aerial parts of the plant unless otherwise stated. The numbers after each alkaloid are the percentage of alkaloid by weight based on the dry weight of the plant.

No references have been found to investigations on the alkaloid content of *D. ceratocaula*, *D. leichhardtii*, *D. discolor* or *D. pruinosa*.

These variations in the alkaloid content are markedly distinct due to different localities, environments and stage of growth (Chaudhuri 1954 ;Griffin 1976 ; Gupta et al. 1973 ; Kapahi and Sarin 1978 ; Karnick and Saxena 1970 ; Leete 1959 ; Trease and Evan 1985). The nitrogen fertilization of the soil can increase the alkaloid yield from D. stramonium, D. metel, D. innoxia and D. discolor (Afridi et al. 1977 ; Fluck and Nisoli 1954 ; Kurowicka - Kuleszyna 1953 ; Leete 1959 ; Madneno Box 1984 ; Mahran et al. 1974 ; Maurin 1925 ;Shibata et al. 1951, 1952) ; but however the ratio of hyoscyamine to hyoscine in a particular species did not change.

Some fifteen species have been studied in chemical details and all exhibited much the same type of alkaloid spectrum ; in this respect, the tree daturas (Brugmansia), sometimes afforded generic rank, exhibit no marked differences to the other sections because a number of botanists did not accept the generic rank given to Brugmansia but considered these plants to be so closely connected with other species of the genus as to form a section of Dutra (Hawkes et al. 1979 ; Leete 1959). The aerial parts of all plants contain hyoscine and/or l-hyoscyamine as principal alkaloids with smaller amounts of derivatives of these bases. The roots contain, in addition, a large numbers of esters formed from tropane-diol and tropane-triol. The alkaloids characterised from the genus are given in Table 2 (p.17) (Hawkes et al. 1979). Although not all the alkaloid listed have been isolated from each species, the variation between species appears largely to be one of

Table 2 Alkaloids of Datura Species

Alkaloid	Basic moiety	Esterifying acid (s)	Occurrence in genus	Reference to isolation from Datura
Tropine			All spp.	Evans & Wellendorf (1959)
Hyoscyamine (and atropine)	Tropine	Tropic acid	All spp.	Feldhaus (1985)
Apoatropine	Tropine	Atropic acid	Limited	Romeike (1953)
Littorine	Tropine	2-Hydroxy-3-phenyl-propionic acid	General	Evans & Major (1968)
3α-Tigloyloxytropane	Tropine	Tiglic acid	General	Evans & Wellendorf (1959)
3α-Acetoxytropane	Tropine	Acetic acid	*D. sanguinea*	Evans & Major (1966)
Hyoscyamine N-oxide (two isomers)	Tropine N-oxide	Tropic acid	*D. stramonium*	Phillipson & Handa (1975)
Norhyoscyamine (and noratropine)	Nortropine	Tropic acid	General	Carr & Reynolds (1912)
Pseudotropine			Most spp.	Evans & Wellendorf (1959)
Tigloidine	Pseudotropine	Tiglic acid	Several spp.	Evans & Wellendorf (1959)
Tropane-3α,6β-diol			General	Evans & Than (1962)
(-)-3α,6β-Ditigloyloxytropane	(+)-Tropane-3α, 6β-diol	Tiglic acid (2 mol)	All	Evans & Wellendorf (1958)
(-)-6β-Tigloyloxy-tropan-3α,ol	(+)-Tropane-3α, 6β-diol	Tiglic acid (2 mol)	*D. innoxia*	Evans & Griffin (1963)
(-)-3α-Tigloyloxy-tropan-6β-ol	(+)-Tropane-3α, 6β-diol	Tiglic acid (2 mol)	*D. innoxia*	Evans & Griffin (1963)
(±)-3α-Tigloyloxy-tropan-6β-ol	(±)-Tropane-3α, 6β-diol	Tiglic acid (2 mol)	*D. suaveolens*	Evans & Lampard (1972)
6β-Acetoxy-3α-tigloyloxytropane	(+)-Tropane-3α, 6β-diol	Tiglic and acetic acids	*D. sanguinea*	Evans & Major (1966)
6β-(2-Methylbutyryloxy)-3α-tigloyloxytropane	(+)-Tropane-3α, 6β-diol	Tiglic+2-methylbutyric acids	*D. suaveolens*	Evans & Lampard (1972)
6β-Propanoyloxy-3α-tigloyloxytropane	(+)-Tropane-3α, 6β-diol	Tiglic + propionic acid	*D. innoxia*	Beresford & Woolley (1974a)
6β-(2-Methylbutyryloxy) tropan-3α,ol	(+)-Tropane-3α, 6β-diol	2-Methylbutyric acid	*D. ceratocaula*	Beresford & Woolley (1974c)
6-Hydroxyhyoscyamine	(-)-Tropane-3α, 6β-diol	Tropic acid	Interspecific hybrids	Romeike (1962a)

Table 2 (continued)

Alkaloid	Basic moiety	Esterifying acid (s)	Occurrence in genus	Reference to isolation from *Datura*
6β-Tigloyloxynortropan-3α-ol	Nortropane-3α, 6β-diol	Tiglic acid	*D. sanguinea*	Unpublished result
Meteloidine	Tropane-3α, 6β,7β-triol	Tiglic acid	Most species	Pyman & Reynolds (1908)
6β-Tigloyloxytropan-3α,7β-diol	Tropane-3α, 6β,7β-triol	Tiglic acid	*D. suaveolens*	Evans & Lampard (1972)
3α,6β-Ditigloyloxytropan-7β-ol	Tropane-3α, 6β,7β-triol	Tiglic acid (2 mol)	All species	Evans & Partridge (1957)
6β-Isovaleryloxy-3α-tigloyloxytropan-7β-ol	Tropane-3α, 6β,7β-triol	Tiglic+isovaleric acids	*D. suaveolens*	Evans & Major (1968)
Hyoscine	Scopine	Tropic acid	All spp.	Kirchner (1905)
Apohyoscine	Scopine	Atropic acid	Most spp.	Evans & wooney (1965)
Hyoscine N-oxide	Scopine N-oxide	Tropic acid	*D. stramonium*	Phillipson & Handa (1975)
Oscine			Tree daturas	Evans & Major (1968)
Norhyoscine	Norscopine	Tropic acid	Most spp.	Evans et al. (1965)
Cuscohygrine			All spp.	van Haga (1954)

degree with minute amounts of some bases often being difficult to separate from the more prevalent components (Hawkes et al. 1979).

4.1) Chemistry of Tropane Alkaloids (Innes et al. 1980 ; Leete 1959) : Apart from cuscohygrine all the alkaloids of *Datura* are derivatives of tropane (I) (Figure 1,p.20). These alkaloids are organic esters formed by combination of an aromatic acid, tropic acid (II), and complex organic base, either tropine (tropanol) (III) or scopine (IV). Scopine differs from tropine only in having an oxygen bridge between the carbon atoms designated as 6 and 7 in the structural formulas. Hyoscine (V) and l-hyoscyamine (VI) are ester of tropic acid and alcohol scopine or tropine respectively. Homatropine is a semisynthetic compound produced by combination of the tropine and mandelic acid. There is an asymmetrical carbon atom in tropic and mandelic acids (boldface C in the formulas in Figure 1,p.20). Hyoscine is l-form and is much more active than d-form. Atropine is racemic form of hyoscyamine occurring during extraction process and consists of mixture of equal parts of d- and l-hyoscyamine but the antimuscarinic activity is almost wholly due to the naturally occurring l-form. L-hyoscyamine is thus twice as potent as atropine in its antimuscarinic activity.

4.2) Tropane Alkaloids of Datura Metel L.var. Fastuosa. Safford

The principal alkaloid of *D. metel* var. *fastuosa* is hyoscine or scopolamine (Punyarajun and Tipduangta 1981 ; Shah and Khanna 1963) with traces of l-hyoscyamine, dl-hyoscyamine

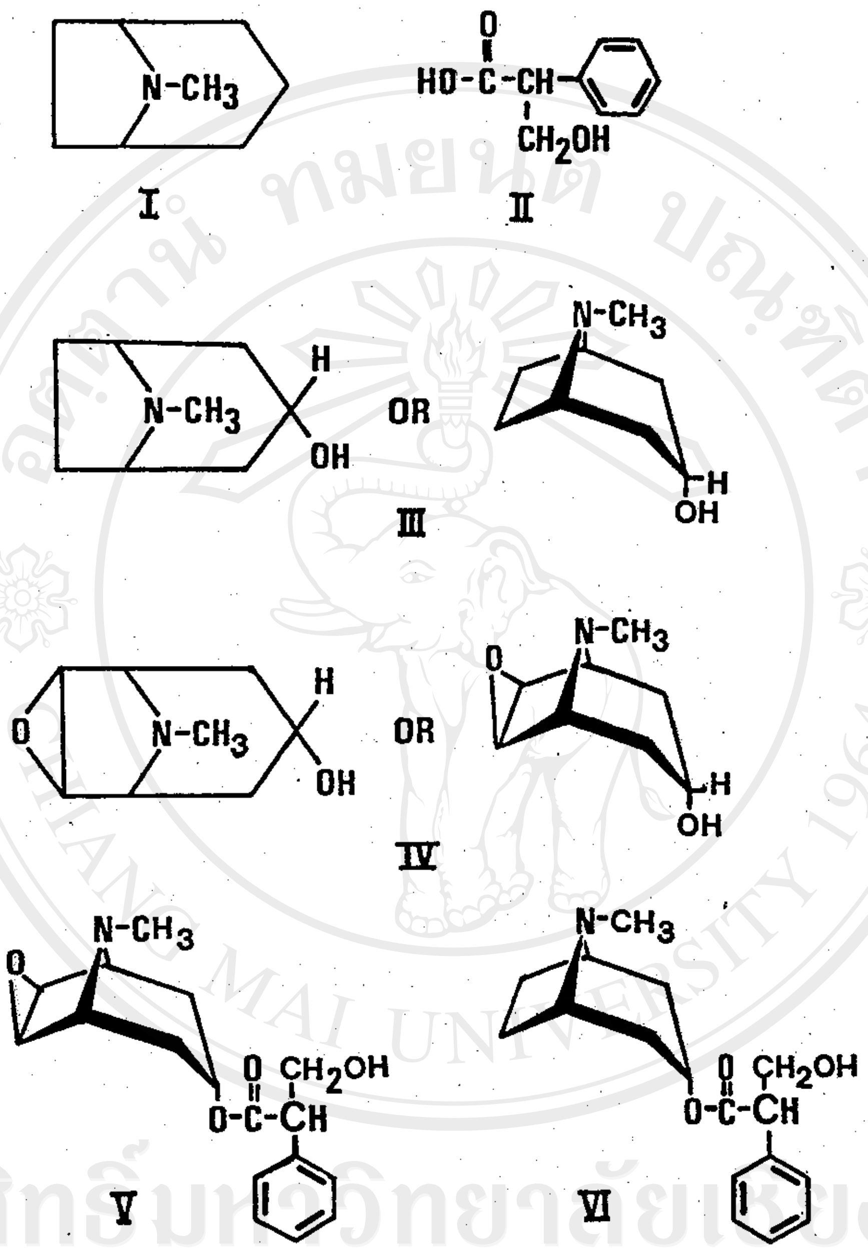

Figure I: Structural Formulas of Tropane (I), Tropic acid (II), Tropanol (III), Scopine (IV), Hyoscine (V) and Hyoscyamine (VI).

(atropine) and norhyoscyamine. The presence of hyoscine at nearly two times of hyoscyamine in all plant parts which are tested at the elevation of 313.13 meters above the average sea level of Chiangmai province (Punyarajun and Tipduangta 1981) and the total alkaloid content in the overground parts ranged from 0.401 percent to 1.964 percent. According to Somanabandhu and Suntorncharoenon (1980), *D. metel* var. *fastuosa* which was collected from Bangkok and Ang Tong provinces showed that hyoscine appeared to be the major alkaloid in the leaves whereas atropine appeared to predominate in the roots. According to Shah and Khanna (1963) observations, leaves of both *D. metel* and *D. metel* var. *fastuosa* contain hyoscyamine and hyoscine 50 and 40 percent of total tropane alkaloids respectively. In entire herb, percentage of hyoscine and hyoscyamine are 50 and 40 percent respectively which may be attributed to the higher percentage of hyoscine in flowers. The total alkaloid content of *D. metel* var. *fastuosa* is about 1.5 times of that of *D. metel*.

Quantitative analysis of the aerial parts and roots of *D. metel* var. *fastuosa* was carried out on samples collected from different locations and the results are shown in Table 3 (p.22).

5) Biosynthesis of Tropane Alkaloids.

5.1) Biosynthesis of Hyoscyamine(Atropine).

The characteristic of tropane alkaloids is ester of hydroxytropanes (the alkaline part) and various acids (the acidic part) (Hawkes *et al*. 1979 ; Trease and Evan 1985; Tyler *et al*.

Table 3 : Percentage of Alkaloids in D. metel var. fastuosa (Calculated on dry weight basis).

Plant	Source	Plant part	Hyoscine fraction (%)	Hyoscyamine or atropine fraction(%)	Total alkaloids (%)	Method*
D. metel L. var. fastuosa	Bangkok-Thailand (Somanabandhu and Suntorncharoenon 1980)	Leaves	-	-	0.10	1
			-	-	0.14	2
			-	-	0.09	1
		Roots	-	-	0.038	1
	Ang Tong-Thailand (Somanabandhu and Suntorncharoenon 1980)	Leaves	-	-	0.09	1
			-	-	0.11	2
	Chiangmai-Thailand (Punyarajun and Tipduangta 1981)	Leaves	-	-	0.501	2
		Flowering Tops	-	-	0.975	2
		Young flowers	-	-	1.167	2
		Blooming flowers	-	-	1.964	2
		Ripe seeds	-	-	0.401	2
	Calcutta-India (Shah and Khanna 1963)	Leaves	0.261	0.330	0.661	1
		Herbs	0.306	0.272	0.637	1
		Flowers	0.477	0.321	0.890	1

* 1 = direct titration
2 = back titration

1981). Most investigations of their biosynthesis have been performed extensively on various species of Datura but all the evidences suggest that similar pathways operate in other tropane alkaloids-producing plants (Trease and Evan 1985 ; Tyler et al. 1981). Feeding studies with labelled ornithine (ornithine-2-^{14}C) have shown that this amino acid is incorporated stereospecifically to form the pyrrolidine ring of tropine (Bernfeld 1967 ; Leete 1959 ; Robinson 1968 ; Sim 1970 ; Swan 1967 ; Trease and Evan 1985 ; Tyler et al. 1981). The remaining three carbon atoms are derived from acetate and thus completed the piperidine moiety (Bernfeld 1967 ; Tyler et al. 1981). Methylation occurs via transmethylation from a suitable donor, for example, methionine, to complete the tropine nucleus (Figure 2. p.24) (Robinson 1968 ; Sim 1970 ; Trease and Evan 1985 ; Tyler 1981).

Phenylalanine is the precursor of tropic acid by an intramolecular rearrangement of the side chain during the conversion (Bernfeld 1967 ; Robinson 1968 ; Sim 1970 ; Trease and Evan 1985 ; Tyler 1981). Tropine on esterification with tropic acid affords hyoscyamine (Figure 2, p.24) from which various alkaloids are derived. During the extraction process, racemization readily occurs since the asymmetric carbon atom is adjacent to a carbonyl group and can enolize. Dl-hyoscyamine resulting from this racemization is known as atropine (Robinson 1968).

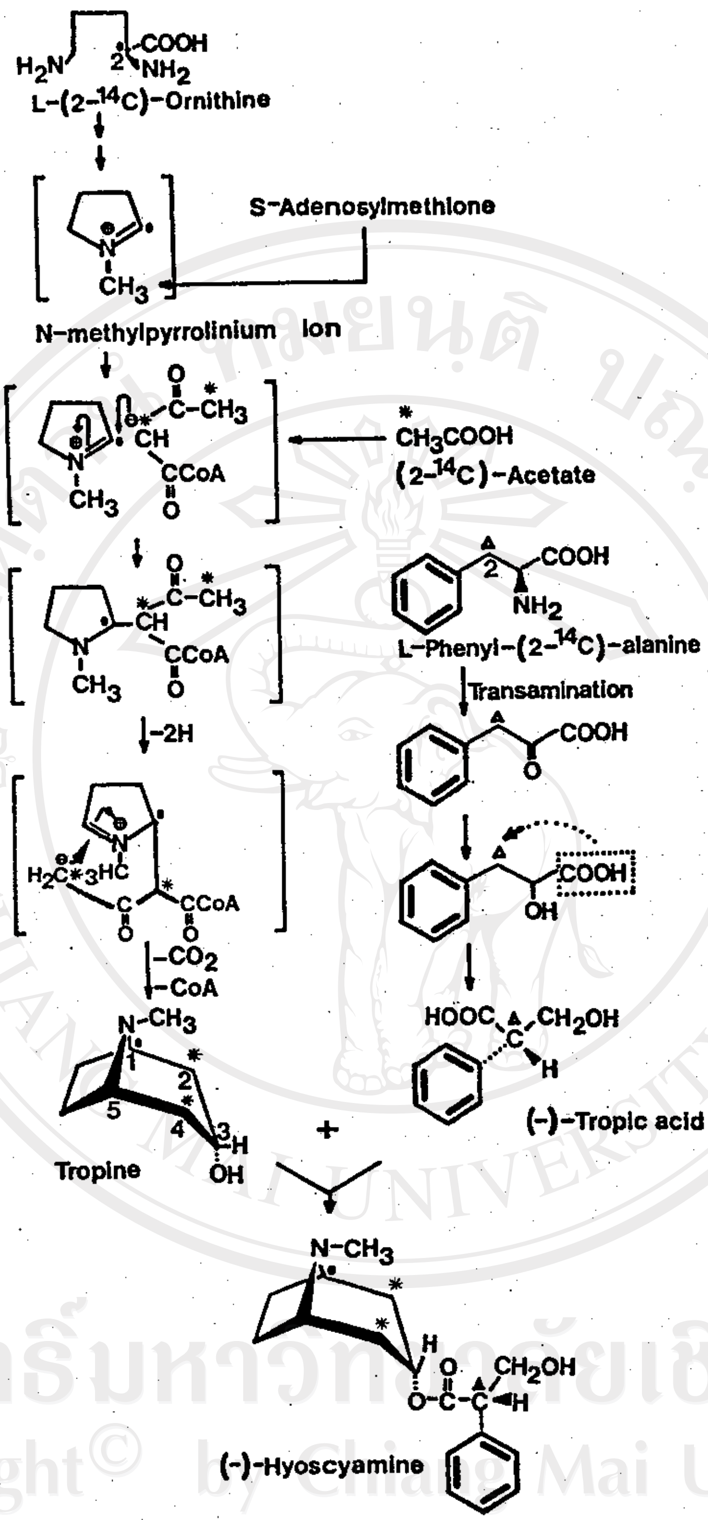

Figure 2. Biosynthesis of Hyoscyamine (Tyler et al. 1981).

5.2) Biosynthesis of Hyoscine.

Hyoscyamine is a precursor of hyoscine and intermediates in this transformation are apparently 6,7-dehydrohyoscyamine and 6-hydroxyhyoscyamine. Epoxidation of the latter leads to hyoscine. These reactions are summerized in Figure 3 (p.26).

6) Extraction and Isolation of Tropane Alkaloids.

In the extraction and isolation of tropane alkaloids from the plant materials, there is often a great problem to separate these alkaloids, which constitute only a small amount, from the bulk of the non-alkaloidal materials. Extraction methods vary with the scale and purpose of the operation (Griffin et al. 1975 ; Hamon and Eyolfson 1972 ; Hikino et al. 1983 ; Pennington and Schmidt 1982 ; Punyarajun 1982 ; Somanabandhu and Suntorncharoenon 1980 ; United States Pharmacopoeia 1979 ; Wu-chu et al. 1969 ; Wyatt et al. 1976) and the procedure most commonly employed is one of the following general methods described below. (Sim 1970 ; Trease and Evan 1985).

Process (A) : The water-immiscible organic solvent is used for the initial extraction of the powdered material. The alkaloids usually occur in plants as salts in complex mixtures of water-soluble compounds and all kinds of lipid, therefore, with plant material, the primary extraction is usually effected with organic solvents after the liberation of the alkaloid bases from their salts by treatment with mineral base (Holmes 1959 ;Sim 1970 ;

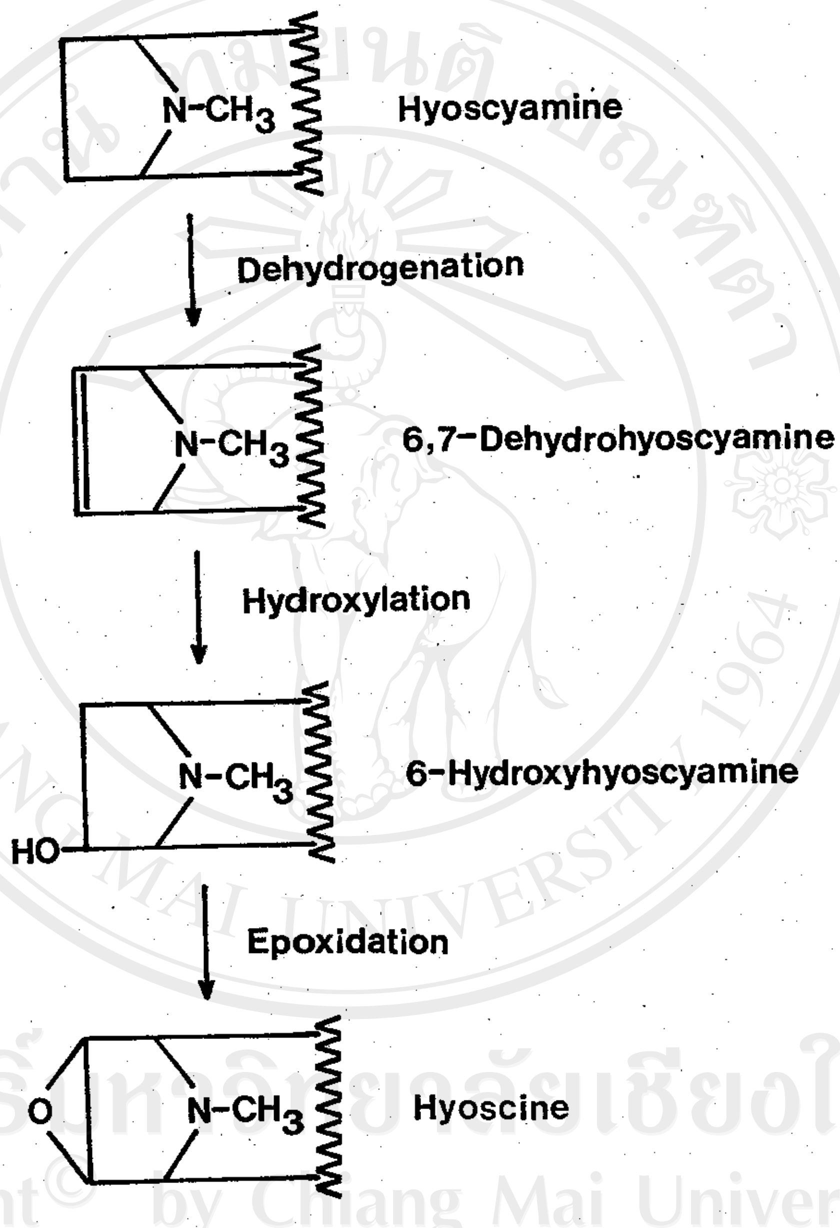

Figure 3. Biosynthesis of Hyoscine (Trease and Evan 1985).

Svendsen and Verpoorte 1983). As prolonged contact with strong bases may lead to alterations to many alkaloids, such as hydrolysis of ester alkaloids, and as strong bases also cause the formation of soaps, if fat are present, ammonia is most commonly use. Ammonia is sufficiently basic to liberate most of the common alkaloids without risk of undesirable reactions. Also, as ammonia is volatile, it can easily be removed afterwards.

Ether and chloroform are among the most commonly used for such extraction (Holmes 1959 ; Sim 1970 ; Svendsen and Verpoorte 1983). The latter solvent offers the advantage that the extract can be drawn from the bottom of the separating funnel, so that the manipulation of large volumes is minimized. The alkaloidal extract presented in organic solvent is then shaken with aqueous acid and allowed to separate. Alkaloidal salts are now in the aqueous liquid, while some undesirable constituents still remain behind in the organic liquid, then remove this organic phase from the aqueous acid solution. Extraction is then carried out in continuously as same as before. The alkalinity converts the alkaloidal salts in the acidic aqueous solution to the free base forms of the alkaloids which are usually much more soluble in the organic solvent, thus these alkaloid bases pass into the organic solvent layer (Holmes 1959 ; Sim 1970 ; Svendsen and Verpoorte 1983 ; Trease and Evan 1985).

When the plant material (for example, seeds) is very rich in fats, removal of these glycerides by preliminary extraction with a petroleum solvent is often advantageous (Holmes 1959).

Process B : The alkaloidal salt in plant materials is extracted by means of water-alcohol mixtures (Sim 1970 ; Svendsen and Verpoorte 1983 ; Trease and Evan 1985). However, the risk of alterations to the genuine alkaloids present in the material is less under such conditions. Extraction with most organic solvents, gives extracts containing all kinds of lipids and resins, whereas extraction made with water and water-alcohol mixtures will give extracts containing various polar compounds, such as proteins, gums and mineral salts (Svendsen and Verpoorte 1983). In this case, after further purification of alkaloidal extracts is achieved by shaking with organic solvent, it is now sufficiently free from pigments and other unwanted materials. Extraction is then carried out by discarding the organic solvent and further extracted as in process (A) or the free alkaloids are then precipitated by the addition of excess of sodium bicarbonate (or ammonia) and separated by filtration or by extraction with organic solvent (Trease and Evan 1985).

7) Quantitative Determination of Tropane Alkaloids.

For the determination of total alkaloids, the method is based on titrimetric technique which is official in United States Pharmacopoeia XVIII (1970). On the principle that only tropane alkaloids are esters of organic acid and hydroxytropane. The determination of total alkaloid contents will be either direct titration or residual titration and calculated in term of the alkaloid hyoscyamine (atropine) (Punyarajun and Tipduangta 1981 ; Punyarajun

1982 ; Shan and Khanna 1963 ; Somanabandhu and Suntorncharoenon 1980). This method has been widely used for a long time. The most common standard acid and base are sulfuric acid and sodium hydroxide. The indicator is usually bromocresol green for direct titration (Karnick and Saxena 1970 ; Shah and Khanna 1963 ; Somanabandhu and Suntorncharoenon 1980) or methyl red for residual titration (Punyarajun and Tipduangta 1981 ; Punyarajun 1982 ; Somanabandhu and Suntorncharoenon 1980).

This official method lacked of sensitivity and specificity (Grady and Zimmerer 1970 ; Padula et al. 1976). Small amounts of alkaloids could not be assayed and decomposition products were not excluded. Numerous methods have been reported for the detection and the determination of tropane alkaloids in plant materials such as paper chromatography (Metwally et al. 1976 ; Padula et al. 1976; Turowska et al. 1972 ; Zimmerer and Grady 1970), partition-column chromatography (Zimmerer and Grady 1970), thin layer chromatography (Carlos 1971 ; Padula et al. 1976 ; Pletneva et al. 1973 ; Saint - Firmin and Paris 1967), thin layer chromatography with densitometry (Grady and Zimmerer 1970 ; Gros-Lebon and Debelmas 1972 ; Massa et al. 1970 ; Padula et al. 1976 ; Wu chu et al. 1969), colorimetry (Bhansali 1972 ; Bracey and Selzer 1968 ; Ganescu et al. 1976 ; Grady and Zimmerer 1970 ; Koch et al. 1965 ; Li, Wenyen and Li, Yongfei 1981 ; Nin'O 1967 ; Wisniewski and Gwiazdzinska 1972), fluorometry (Messerchmidt 1969 ; Robert 1969), infrared spectrophotometry (Padula et al. 1976) and ultraviolet-visible spectrophotometry (Gomaa and Taha 1975 ; Hassan and Davidson 1984 ;

Minamikawa and Yamagishi 1973 ; Woodson 1970 ; Zimmerer and Grady 1970). Some workers have explored the gas chromatography of tropane alkaloids. Because of its specificity, sensitivity and good resolving properties, gas liquid chromatography (GLC) has been increasingly used for tropane alkaloids analysis. It is a sophisticated and efficient separation technique for the analytical laboratory (Santoro and Zarembo 1979). The USP collaborative study for the assay of atropine and hyoscine dosage forms by gas chromatography indicated that these two substances could be assayed in this manner with an acceptable degree of precision and is the basis for official methods in USP. However, the case becomes less clear, even with the high resolving power of gas chromatography, when found in the presence of preponderant concentration of other amines with high background contributions. The problem is further complicated when both atropine and hyoscine must be determined together. The procedure have covered many types of chromatographic conditions as shown in Table 4 (p.31).

High pressure liquid chromatography (HPLC) has, in common with gas chromatography, specificity, sensitivity and good resolving power. The use of HPLC in the separation and the determination of tropane alkaloids has been documented (Brown and Sleeman 1978 ; Honigberg et al. 1975 ; Pennington and Schmidt 1982; Santi et al. 1975 ; Santoro and Zarembo 1979 ; Stutz and Sass 1973; Walters 1978 ; Verpoorte and Svendsen 1974, 1976).

Table 4 : Summarization of Tropane Alkaloids Determination by GLC

Name of worker (s)	Sample	Stationary phase	Column Type	Column Size	Temperature (°C) Oven	Temperature (°C) Injector	Temperature (°C) Detector	Mobile phase (ml/min)	Type of detector	Derivatisation	Internal standard	Substances* detected
Grady, L.T. and Jr. Zimmerer, R.O. (1970)	atropine and hyoscine dosage froms	3% OV-17 on 80/100 or 100/120 mesh Gas Chrome Q	glass	0.6mx3mm or 1.2mx 3mm	210° or 215°	not more than 25° above of the column	not more than 25° above of the column	He, 60	FID	-	homatropine	A,H
Brochmann-Hanssen E.and Svenden, A.B. (1962)	alkaloids and alkaloidal derivatives	1.15% SE-30 on Gas Chrome P 100-140 mesh	glass	6ftx3mm	175°	325°	325°	N_2, 25	FID	-	-	A,H and others
Santoro, R.S. et al. (1973)	belladonna alkaloids in dosage forms	3% OV-17 on Gas Chrome Q 100-120 mesh	glass	4ftx4mm	210°	not more than 25° above of the column	not more than 25° above of the column	He, 50	FID	-	homatropine HBr USP. reference standard	A,H
Windheuser, J.L. et al. (1972)	hyoscine and its degradation products	1% OV-17 on Gas Chrome Q 80/100 mesh	glass	2.4mx4mm	isothermal at 100 for 13 min, then 10/min. rise up. to 220° and then isothermal at 220	240°	340°	N_2, 70	FID	silylation(N,O bis (trimethyl-silyl) acetamide	N,N-dimethyl formamide	H,Scopoline, Atropic acid, Tropic acid, Aposcopolamine
Solomon, M.J. et al. (1969)	plant Powders of A. belladonna and D. stramonium	2.5% SE-30/5 on Chromosorb G 80-100 mesh	glass	6ftx0.875 inch	programmed 150-250° at 6/min	315°	315°	He, 99.5 ±0.5	FID	-	-	A,H,Ap
Wu Chu, B.L. and Mika, E.S. (1970)	Hyoscyamus powders	2.5% SE-30 on Chromosorb G 80-100 mesh	glass	1.83mx 0.19cm	isothermal at 220° for 6 min, followed by programmed temp. from 200-290°	300°	300°	He, 100	FID	-	tetraphenyl ethylene	A,H

Table 4 (continued)

Name of worker (s)	Sample	Stationary phase	Column		Temperature (°C)			Mobile phase (ml/min)	Type of detector	Derivatisation	Internal standard	Substances detected*
			Type	Size	Oven	Injector	Detector					
Wyatt, D.K., et at. (1976)	belladonna pilular and powdered extract and tincture	3% OV-17 on Gas Chrome Q 100-120 mesh	glass	1.2mx4mm	215°	240°	245°	He, 65	FID	-	homatropine	A,H,Ap
Griffin, W.J. et al. (1975)	Duboisia myoporoides and D. leichhardtii	1.5% SE-30 on Chromosorb W 80-100 mesh	glass	150cmx4mm	191°	244°	244°	He, 190	FID	silanized with either hexamethyldisilazane or N,O-bis(trimethylsilyl)-acetamide	homatropine	A,H and
Padula, L.Z. et al. (1976)	roots, stems, leaves, fruits and seeds of Datura ferox	3% OV-17 on Gas Chrome Q 100-120 mesh	glass	2.0mx4mm	240° (corrected)	290°	290°	N_2 40	FID	-	-	H
Mechler E. and Kohlenbach, H.W. (1978)	the diploid plants of Datura innoxia Mill., D. meteloides Dun. and D. wrightii Regel.	3% OV-17 on Gas Chrome Q	glass	4ftx0.25 inch	235°	250°	300°	He 30	FID	-	-	M,A,H
Parker,K.D. et. al. (1963)	toxicological extracts	5% SE-30 on Chromosorb W 60-80 mesh	glass	5ftx0.093 inch	210°, 230°, 250° or 270°	30° above oven temp.	30° above oven temp.	N_2,30.7	FID	-	-	A,H,Ho and others

* A = Atropine ; Ap = Apoatropine ; H = Hyoscine ; M = Meteloides ; Ho = Homatropine

The technique used in this thesis is based on the GLC method to determine medicinally important tropane alkaloids, atropine-hyoscyamine, hyoscine and its degradation products.

8) Medicinal Properties and Uses.

In traditional medicine, leaves, seeds and roots of Datura metel var. fastuosa, are used.(Maeinvongyati 1986 ; Tantivat 1978). These parts of the plant which possess narcotic, anodyne andantispasmodic properties are useful in neuralgia and antispasmodic (Quisumbing 1951 ; Sarin 1982).

Datura possesses properties analogous to those of Belladonna ; when locally applied in aqueous solution, it causes dilation of the pupil equal to that caused by atropine solution at the dilution of 1 to 120 (Quisumbing 1951). In traditional medicine of all over the East, the leaves and the flowers are cut in small pieces and smoked, like tobacco, in a pipe. It can relieve the attack of bronchial asthma (Maeinvongyati 1986 ; Perry et al. 1980 ; Quisumbing 1951 ; Tantivat 1978). They are also applied as an anodyne poultice to inflammed breasts in order to reduce the inflammation due to excessive secretion of milk during lactation period (Quisumbing 1951 ; Tantivat 1978). The Malays use the leaves in the treatment of boils, sores on the legs, haemorrhoids, rheumatism, swollen joints and fishbites as well as for ringworm and sorethroat. Fresh juice of leaves is also useful in earache, 1-2 drops are being applied into the ear.

The leaves boiled in oil is a good application to haemorrhoid anal fissures and other diseases of the rectum leading to tenesmus (Perry *et al*. 1980 ; Quisumbing 1951). This juice is also a popular internal remedy for the prevention of rabies. The powdered roots are rubbed on the gum for toothache. The seeds pounded in oil are used as an embrocation in rhumatism and applied to syphilitic swellings and boils (Quisumbing 1951).

In modern medicine (Leete 1959; Innes and Nickerson 1980 ; International Trade Center UNTAD/GATT 1982 ; Sarin 1982 ; Tyler *et al*. 1981), *Datura* acts chiefly by virtue of its alkaloid contents, hyoscine and hyoscyamine. They are of considerable pharmaceutical interests because of their parasympatholytic, anticholinergic, antiemetic and sedative actions. Hyoscine resembles atropine in its peripheral action but differs greatly in its central effects ; it is a primary central depressant with sedative and tranquilizing properties. Like atropine, it is a potent mydriatic and has cycloplegic effect which use to prevent adhesions between iris and lens of the eye in case of iritis. In recent years it has been found increasing use in relief of withdrawal symptoms in morphine and alcohol addiction and in abnormal sexual excitement. Clinically useful effects of hyoscine butylbromide obtained from blocking the muscarinic activity of acetylcholine are an antispasmodic effect used principally to relieve spasms of bowel in treatment of spastic colitis, gastroenteritis and peptic ulcer ; and antisecretory effect used to reduce anesthesia , gastric secretions in peptic ulcer therapy and nasal and sinus secretions in common cold and allergy medication, and the methobromide salt may be used in the similar way. Recent studies have suggested that transdermal administration of the latter may be useful in maintenance treatment of duodenal ulcer. Absorption of the drug through skin may reduce side effects that limit the use of anticholinergics in duodenal ulcer. The hydrobromide salt is used as sedative in the treatment of acute mania in pre-operative medication and in the preparations for motion sickness.

Atropine sulfate is an antidote in case of poisoning caused by cholinesterase inhibitors such as physostigmine and organophosphate insecticides.

9) Objectives.

1) to study the influence of fertilizers on total alkaloid production and on the amount of hyoscyamine and/or atropine and hyoscine in various parts of D. metel L. var. fastuosa Safford.
2) to study the appropriate stage for harvesting different parts of D. metel L.var. fastuosa Safford so as to obtain high total alkaloid contents, hyoscyamine and/or atropine and hyoscine.
3) to adapt the method for analysing hyoscyamine and/or atropine and hyoscine contents in different parts of D. metel L. var. fastuosa Safford.