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The Value of the Duquénois Test for Cannabis—A Survey

The analytical procedures employed in drug identification have come under increasing scrutiny in recent years. Since cannabis is one of the most commonly found illicit drugs, an extra focus has been brought on its identification. Thus, several publications [*1-5*] have outlined methods of attacking the testimony of analysts who have identified cannabis for forensic purposes.

The term "cannabis" will be employed in this article to denote a preparation of *Cannabis*, such as marihuana or hashish, which contains cannabinoids. *Cannabis* comprises the species *Cannabis sativa* L. and its variants (which are regarded by a minority of plant taxonomists as individual species of *Cannabis*) [*6*]. "Cannabinoids" is meant to include compounds of synthetic or natural origin from *Cannabis* whose structure comprises a 5-alkylresorcinol moiety covalently bonded to a monoterpenoid or analogous moeity [*7*], such as cannabidiol (CBD), synhexyl (para- or pyra-hexyl), and tetrahydrocannabinol (THC) (Fig. 1).

The Duquénois test is one of the best-known color tests used as an aid in drug identification since it is employed throughout the world as part of the procedure for identifying cannabis. According to Duquénois [*8*], the acetaldehyde-vanillin reaction was proposed in 1937 in collaboration with H. Negm (Hassan Negm Mustapha, cited variously as Negm or Mustapha) and was adopted by the League of Nations Subcommittee on *Cannabis* in 1939. Publications on its use appeared in 1938 [*9,10*]. The test in original and modified forms has subsequently been applied to many materials in attempts to assess its specificity. The results depend on a subjective opinion of the similarity of the color developed with the test material to that developed with cannabis, and "false positives" have been claimed. It is exceedingly difficult to describe colors and hues in a few words, especially in cases where they are undergoing continuous changes. Thus, while experienced analysts have an excellent concept of the color developments, these are difficult to describe. Nevertheless, the experienced analyst can confidently decide if the similarly described colors perceived from test substances are distinguishable from those from cannabis. This article therefore endeavors to assemble and review published data and set Duquénois tests in context for an objective evaluation. It is hoped that forensic analysts will find the compilation and documentation useful.

Duquénois Tests

The Duquénois reagent consists of vanillin (0.4 g) and acetaldehyde (0.06 g) dissolved in 95% alcohol (20 ml) [*8-10*]. The procedure was to pour 2 ml of reagent onto the petroleum ether extract (preferably still warm) in an evaporating dish, stir to complete

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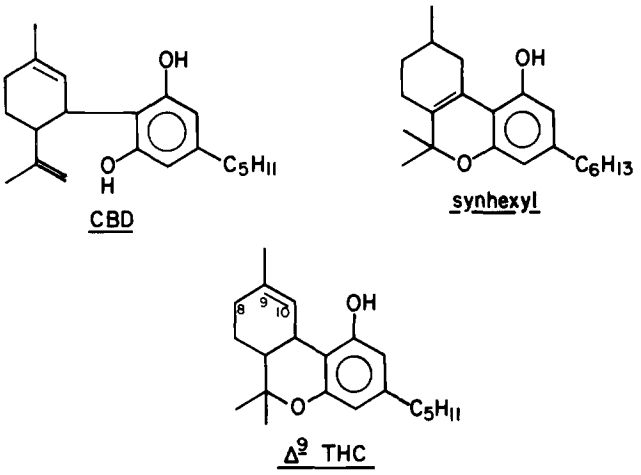

FIG. 1—*Structures of cannabidiol* (CBD), *synhexyl, and tetrahydrocannabinol* (THC).

solution, and add 2 ml concentrated hydrochloric acid. Duquénois originally stated that the reaction is specific if one considers the succession of tints (for cannabis: sea-green, slate, followed by indigo within 10 min; within ½ h the color turns to violet and within an hour to intense violet). This will be referred to as the D-N test.

Butler [*11*] reported the modified Duquénois test in 1962. Known as the Duquénois-Levine test, it was introduced in 1941. The important change is to incorporate extraction of the color into chloroform (2 ml), which is added 10 min after the addition of the hydrochloric acid. Butler's paper is interesting in that seven collaborators examined eleven samples of marihuana, mixtures, and interfering substances by the Duquenois-Levine test alone and none of the samples gave a false positive test. A test of this type, which includes a preliminary extraction with hexane or petroleum ether and partition of the color between chloroform and water, will be referred to as a D-L test.

The "rapid Duquénois" test [*12,13*] (it would have been better termed "rapid Duquénois-Levine" test) omits the petroleum ether extraction stage, that is, the reagent is added directly to the suspect material in a test tube and the chloroform is added after the color transitions have been noted. Tests of this type will be termed RD-L tests.

The "meta-Duquénois" test [*13*] employs a solid reagent of 1% metaldehyde in vanillin added (100 mg) to the suspect sample (1 to 2 mg, no extraction stage) in a test tube and heated with ethanol (1 ml) for a few seconds to bring about solution. Concentrated hydrochloric acid (1 to 3 ml) is added to the hot solution, the color noted, chloroform (1 to 3 ml) added, and the partition of color between the phases noted. This will be termed an MD-L test.

As will be shown, the various tests have different specificities, the best being the D-L type.

Duquénois Tests Applied to Herbal or Botanical Materials

Published results of the Duquénois testing of various herbal or herbally derived materials are assembled in Table 1. The plant names employed by the original author are used and therefore there is a certain amount of (valuable) duplication, such as, *Humulus lupulus* and hops. When the author has provided two names (the Latin binomial and the common English name), the English name has usually been employed because Maunder's lengthy list [*13*] employs English names and using that for a start enables a more ready comparison

TABLE 1—*Results obtained with various herbal materials (excluding cannabis) in the Duquénois test.* [a]

Test Material	Cross Reference	RD-L A	RD-L B	MD-L [13] A	MD-L [13] B	D-N	D-L A	D-L B
Acanthus mollis	...	...	...	...	...	− [14]	...	...
Aconite	...	...	...	−	−	...	...	...
Adenanthera pavonina	sandal	...	...	...	...	...	−	− [14]
Agathis australis	gum copal	...	...	...	...	...	+	− [14]
Agrimony	...	+	− [13]	+	−	...	−	− [13, 18]
Ailanthus	...	...	...	−	−	...	...	...
Alfalfa	...	...	...	...	...	...	...	− [24]
Almond oil/extract	amygdalus oil	−	− [16]	...	...	...	...	...
Aloes	...	...	...	−	−	...	...	...
Amygdalus oil	almond oil	...	...	...	...	− [17]	...	...
Anchusa officinalis	...	...	...	...	...	− [14]	...	...
Angelica	*Angelica archangelica*	...	...	−	−	...	...	...
Angelica archangelica	angelica	...	...	...	...	− [15]	...	...
Anise	anise oil	...	...	−	−	...	...	...
Anise oil/extract	anise	+	+ [16]	...	...	− [10]	?+	− [18]
Annatto	...	...	...	−	−	...	...	...
Aphananthe aspera	...	...	...	...	...	− [14]	...	...
Apocynum	...	...	...	+	−	...	...	...
Arariba	...	...	...	−	−	...	...	...
Armoracia lapathifolia	horseradish	...	...	...	...	− [15]	...	...
Artemisia absinthium	wormwood	...	...	...	...	− [15]	...	...
Artemisia dracunculus	wormwood	...	...	...	...	+ [15]	...	− [14]
Asarum canadense	ginger	...	...	...	...	...	−	− [14]
Asafetida	...	...	...	−	−	...	...	...
Asperugo procumbens	...	...	...	−	−	...	...	...
Atropa belladonna	belladonna	...	...	...	...	− [15]	...	...
Avens herb	...	...	...	−	−	...	...	...
Balm herb	*Melissa officinalis*	...	...	−	−	...	...	...
Balmony herb	...	...	...	−	−	...	...	...
Balsam	...	...	...	...	...	...	...	− [24]
Balsam (Canada gum)	...	...	...	...	...	− [17]	...	...
Banana (dried)	...	...	...	−	−	...	...	...

TABLE 1—*Continued.*

Test Material	Cross Reference	RD-L		MD-L [13]		D-N	D-L	
		A	B	A	B		A	B
Banana (skin)	...	...	...	−	−	...	...	...
Barberry root	...	...	...	−	−	...	...	...
Basil	...	...	...	−	−	...	...	− [24]
Bay oil	...	...	...	...	...	− [17]	...	...
Bayberry	...	...	...	−	−	...	...	...
Bayberry bark	...	...	...	+	−	...	...	...
Bearberry	...	...	...	−	−	...	...	...
Belladonna	*Atropa belladonna*	...	...	−	−	...	...	...
Betel nuts	...	...	...	+	−	...	...	...
Betony	*Pedicularis canadensis*	...	...	...	...	...	−	− [18]
Blackberry leaves	...	...	...	−	−	...	...	...
Black haw bark	...	...	...	−	−	...	...	...
Bladder wrack	...	...	...	−	−	...	...	...
Blessed thistle	...	...	...	−	−	...	...	...
Blumenbachia insignis	...	...	...	...	...	−	...	...
Blumenbachia urens	...	...	...	...	...	−	...	...
Boehmeria nivea	...	...	...	...	...	−	...	...
Boldo	...	...	...	+	−	...	...	...
Boneset	...	...	...	−	−	...	...	...
Borage	...	...	...	...	...	...	−	− [24]
Borago officinalis	...	...	...	...	...	− [14]	...	...
Broom	...	...	...	−	−	...	...	...
Broussonetia kaempferi	...	...	...	...	...	− [14]	...	...
Broussonetia papyrifera	...	...	...	...	...	− [14]	...	...
Bryonia dioica	bryony	...	...	...	...	− [14]	...	...
Bryony	*Bryonia dioica*	...	...	−	−	...	...	...
Buchu	...	...	...	−	−	...	...	...
Buckthorn flowers	...	...	...	...	...	...	+	− [24]
Bugleweed	...	...	...	−	−	...	...	...
Burdock	...	...	...	−	−	...	...	...
Burnet herb (greater)	...	...	...	−	−	...	...	...
Butternut bark	...	...	...	−	−	...	...	...

Cajeput oil/extract	...	+	− [16]	...	...	− [17]	...	...
Cajophora laterita	...	...	...	...	...	− [14]	...	...
Calamus	*Calamus draco*	...	...	+	+	...	...	...
Calamus draco	calamus	...	...	...	...	...	−	− [14]
Calceolaria racemis	...	...	...	...	...	− [14]	...	...
Callitris quadrivalvis	gum sandarac	...	...	...	...	...	+	− [14]
Calumba	...	...	...	−	−	...	...	...
Campanula americana	...	...	...	...	...	− [14]	...	...
Camphor oil/extract	*Cinnamomum camphora*	+	+ [16]	...	...	...	−	− [18]
Candlenut oil	...	...	...	...	...	− [17]	...	...
Canella alba	cinnamon	...	...	+	−	...	...	...
Caplafer conjugata	...	...	...	...	...	...	+	− [14]
Capsicum	...	...	...	−	−	...	...	...
Caraway	caraway oil	...	...	−	−	...	−	− [18]
Caraway oil/extract	caraway	+	+ [16]	...	...	...	−	− [18]
Cardamom	cardamom oil	...	...	+	−	...	−	− [18]
Cardamom oil/extract	cardamom	+	+ [16]	...	...	...	−	− [18]
Cascara	...	...	...	+	−	...	...	...
Castor oil/extract	...	+	− [16]	...	...	− [17]	...	...
Cassia bark	cassia oil	...	...	+	−	...	...	...
Cassia oil/extract	cassia bark	−	− [16]	...	...	...	...	...
Catechu (black)	...	...	...	+	−	...	...	...
Catechu (pallid)	...	...	...	+	−	...	...	...
Catnip	...	−	− [20]	...	...	...	+	− [10,24]
Cedarwood oil	...	...	...	...	...	− [17]	...	...
Celtis occidentalis	...	...	...	...	...	− [14]	...	...
						+ [25]	+	− [25]
Chamole	...	...	...	−	−	...	...	...
Chenopodium oil	...	...	...	...	...	− [17]	...	...
Chervil	...	...	...	−	−	...	...	...
Chicory	...	...	...	−	−	...	...	...
Cinchona bark	...	...	...	+	−	...	...	...
Cinnamon	*Canella alba*	...	...	+	−	...	...	...
Cinnamomum camphora	camphor	...	...	...	...	− [15]	...	...
Cistus	...	...	...	−	−	...	...	...
Citronella oil/extract	...	+	− [16]	...	...	− [17]	...	...
Cleavers herb	...	...	...	−	−	...	...	...
Clove	clove oil	...	...	−	−	...	−	− [18]
Clove oil/extract	clove	+	+ [16]	...	...	− [17]	+	− [18]

TABLE 1—*Continued.*

Test Material	Cross Reference	RD-L A	RD-L B	MD-L [13] A	MD-L [13] B	D-N	D-L A	D-L B
Coca	...	...	...	−	−	...	...	...
Cocillana bark	...	...	...	+	−	...	+	− [14]
Cocoa	...	...	...	+	−	...	...	...
Coconut oil	...	...	...	...	...	− [17]	...	...
Coffea arabica	coffee (green)	...	...	...	...	...	+	− [14]
Coffee (green)	coffee (instant)	...	...	+	+	...	...	...
Coffee (instant)	coffee (roasted)	...	...	−	−	...	+	+ [18]
Coffee (roasted)	*Coffea arabica*	...	...	+	+	...	...	...
Cohosh (blue)	...	...	...	−	−	...	...	...
Colchicum	...	...	...	−	−	...	...	...
Colocynth	...	...	...	−	−	...	...	...
Colophony	pine oil	...	...	−	−	...	...	...
Colophony resin	pine oil	...	...	...	...	− [17]	...	...
Coltsfoot leaves	...	...	...	−	−	...	...	...
Comfrey	*Symphytum officinale*	...	...	−	−	...	...	...
Cordia gerascanthus	...	...	...	...	...	− [14]	...	...
Cordia pubescen	...	...	...	...	...	− [14]	...	...
Coriander	coriander oil	...	...	−	−	...	...	...
Coriander oil/extract	coriander	+	− [16]	...	...	...	−	− [18]
Corn oil/extract	...	?+	− [16]	...	...	...	...	...
Cotton oil/extract	cottonseed oil	+	− [16]	...	...	...	...	...
Cottonseed oil	cotton oil	...	...	...	...	− [17]	...	...
Cramp bark	...	...	...	+	−	...	...	...
Cranesbill herb	...	...	...	−	−	...	...	...
Crossandra undulaefolia	...	...	...	...	...	− [14]	...	...
Croton oil	...	...	...	...	...	− [17]	...	...
Cubeb oil/extract	...	+	− [16]	...	...	...	...	...
Cucumis sativus	...	...	...	...	...	− [14]	...	...
Culver's root	*Leptandra virginica* (*Veronicastrum virginicum*)	...	...	+	+	...	...	...
Cumin oil/extract	cummin	−	− [16]	...	...	...	−	− [18]

Cummin (cumin)	cumin oil	...	...	−	−	...	−	− [18]
Currant	...	...	...	...	...	...	−	− [18]
Curry powder	...	...	...	−	−	...	...	...
Damiana	...	...	...	−	−	...	...	...
Dammar resin	...	...	...	...	...	− [17]	...	...
Dandelion (roasted)	...	...	...	−	−	...	...	...
Date pits (roasted)	...	...	...	−	−	...	...	...
Datura stramonium	stramonium	...	...	...	...	− [15]	...	...
Deertongue leaves	...	...	...	−	−	...	...	...
Derris	derris resin	...	...	+	−	...	...	...
Derris resin	derris	...	...	...	...	− [17]	...	...
Digitalis	...	...	...	−	−	...	...	...
Dill	dill oil	...	...	−	−	...	...	...
Dill oil/extract	dill	+	− [16]	...	...	...	...	...
Dorema ammoniacum	...	...	...	...	...	...	−	− [14]
Dorstenia contrayerva	...	...	...	...	...	− [14]	...	...
Dragon's blood	...	...	...	−	−	...	...	...
Echballium elaterium	...	...	...	...	...	− [14]	...	...
Elder oil/extract	...	+	− [16]	...	...	...	...	...
Elecampane	...	...	...	−	−	...	...	...
Elm leaf	...	...	...	...	...	...	...	− [24]
Ephedra	...	...	...	+	−	...	...	...
Ergot	...	...	...	+	+	...	...	...
Eucalyptus	*Eucalyptus globulus*	...	...	...	...	...	−	− [18]
Eucalyptus globulus	eucalyptus leaves	...	...	...	...	+ [15]	...	− [14]
Eucalyptus leaves	eucalyptus oil	...	...	...	...	...	+	− [11]
Eucalyptus oil	eucalyptus	...	...	...	...	− [17]	...	...
Euphrasia officinalis	...	...	...	...	...	+ [14]	+	− [14]
Fatoua japonica	...	...	...	...	...	− [14]	...	...
Fennel	fennel oil	...	...	−	−	...	...	...
Fennel oil/extract	fennel	+	+ [16]	...	...	− [10]	−	− [18]
Fenugreek	...	...	...	−	−	...	...	...
Ficus carica	other *F.* species	...	...	...	...	+ [15]	...	− [14]
Ficus elastica	other *F.* species	...	...	...	...	+ [14] − [15]	+	− [14]
Ficus repens	other *F.* species	...	...	...	...	+ [14]	+	− [14]
Figwort	...	...	...	−	−	...	...	...
Filipendula ulmaria	...	...	...	...	...	− [15]	...	...
Fumitory	...	...	...	−	−	...	...	...

TABLE 1—*Continued.*

Test Material	Cross Reference	RD-L A	RD-L B	MD-L [13] A	MD-L [13] B	D-N	D-L A	D-L B
Gambage	...	...	...	−	−	...	...	...
Gentian	...	...	...	−	−	...	...	...
Ginger	*Asarum canadense*	...	...	+	+	...	−	− [18]
Ginseng	...	...	...	+	−	...	...	...
Glecoma hederaceum	...	...	...	...	...	− [15]	...	...
Gloxinia antirrhina	...	...	...	...	...	− [14]	...	...
Glycyrrhiza glabra	licorice	...	...	...	...	...	−	− [14]
Goldenseal	...	...	...	−	−	...	...	...
Grass	...	...	...	−	−	...	...	...
Grindelia herb	...	...	...	−	−	...	...	...
Gronovia scandens	...	...	...	...	...	− [14]	...	...
Guaiacum gum	...	...	...	−	−	− [17]	...	...
Gum ammoniac	...	...	...	+	+	...	...	...
Gum animi	*Proteum icicariba*	...	...	+	+	...	...	...
Gum arabic	...	...	...	−	−	...	...	...
Gum barbary	...	...	...	−	−	...	...	...
Gum bdellium	...	...	...	+	−	...	...	...
Gum benzoin (gum benjamin)	...	...	...	−	−	...	...	...
Gum copal	gum kauri	...	...	+	+	...	...	...
Gum galbanum	...	...	...	+	+	...	...	...
Gum ghatti	...	...	...	−	−	...	...	...
Gum karaya (sterculia gum)	...	...	...	−	−	...	...	...
Gum kauri	*Hymenaea courbaril*	...	...	+	+	...	...	...
Gum kino	...	...	...	+	−	...	...	...
Gum myrrh	*Myrrhis odorata*	...	...	+	+	...	...	...
Gum olibanum (frankincense)	gum thus	...	...	+	−	...	...	...
Gum sandarac	*Callitris quadrivalvis*	...	...	+	...	...	...	...
Gum thus	Gum olibanum (frankincense)	...	...	+	−	...	...	...
Gum tragacanth	...	...	...	−	−	...	...	...
Gurjun balsam	...	...	...	...	...	− [17]	...	...
Hellebore (black)	...	...	...	−	−	...	...	...

Henna	*Lawsonia inermis*	−	− [*13*]	+	+	...	− [*13*]	− [*18*]
Holy thistle herb	...	...	...	−	−	...	...	...
Hops	hops (*Humulus japonica*)	...	...	−	−	...	...	− [*24*]
Hops (*Humulus japonica*)	*Humulus lupulus*	...	...	...	...	...	−	− [*18*]
Horehound	...	...	...	−	−	...	...	...
Horseradish	*Armoracia lapathifolia*	...	...	−	−	...	...	...
Horsetail	...	...	...	−	−	...	...	...
Humulus lupulus (flowerbuds)	*H. lupulus* (leaves)	...	...	...	...	+ [*25*]	+	− [*25*]
Humulus lupulus (leaves)	lupulin	...	...	...	...	− [*25*]	...	...
Hydnocarpus oil	...	...	...	...	...	− [*17*]	...	...
Hydrophyllum capitatum	...	...	...	...	...	− [*14*]	...	...
Hymenaea courbaril	kauri resin	...	...	...	...	...	−	− [*14*]
Hyoscyamus	*Hyoscyamus niger*	...	...	−	−	...	...	...
Hyoscyamus niger	hyoscyamus	...	...	...	...	− [*15*]	...	...
Hyssop	*Hyssopus officinalis*	...	...	−	−	...	...	...
Hyssopus officinalis	hyssop	...	...	...	...	− [*15*]	...	...
Indian tobacco flower	lobelia	...	...	−	−	...	...	...
Ipecac	...	...	...	−	−	...	...	...
Iris florentina	*Iris versicolor*	...	...	...	...	...	−	− [*14*]
Iris versicolor	orris	...	...	...	...	...	−	− [*14*]
Jaborandi	...	...	...	−	−	...	...	...
Jalap	...	...	...	−	−	− [*10*]	...	...
Jasmin root	...	...	...	−	−	...	...	...
Juniper oil/extract	*Juniperus communis*	+	− [*16*]	...	...	...	...	...
Juniperus communis	*J. oxycedrus*	...	...	...	...	− [*15*]	...	...
Juniperus oxycedrus	*J. sabina*	...	...	...	...	− [*15*]	...	...
Juniperus sabina	savin	...	...	...	...	− [*15*]	...	...
Kamala	...	...	...	−	−	...	...	...
Kat	...	...	...	−	−	...	...	...
Kauri resin	*Agathis australis*	...	...	...	...	− [*17*]	...	...
Kola	...	...	...	+	−	...	...	...
Labernum	...	...	...	−	−	...	...	...
Lactuca sativa	lettuce opium	...	...	...	...	...	−	− [14]
Lamium album	...	...	...	...	...	− [*15*]	...	...
Lantana camara	...	...	...	...	...	− [*14*]	...	...
Larix decidua	...	...	...	...	...	− [*15*]	...	...
Laurus nobilis	...	...	...	...	...	− [*15*]	...	...
Lavender	lavender oil	...	...	−	−	...	...	− [*24*]

TABLE 1—*Continued.*

Test Material	Cross Reference	RD-L A	RD-L B	MD-L [13] A	MD-L [13] B	D-N	D-L A	D-L B
Lavender oil	*Lavendula aurigerana*	...	...	...	− [17]	...	...	...
Lavendula aurigerana	*L. dentata*	...	...	...	...	− [14]	...	...
Lavendula dentata	*L. officinalis*	...	...	...	...	− [14]	...	...
Lavendula officinalis	*L. pubescens*	...	...	...	...	− [14,15]	...	...
Lavendula pubescens	*L. tarifolia*	...	...	...	...	− [14]	...	...
Lavendula tarifolia	lavender	...	...	...	...	− [14]	...	...
Lawsonia inermis	henna	...	...	...	...	...	−	− [14]
Lepidium sativum	...	...	...	...	...	− [15]	...	...
Leptandra virginica (*Veronicastrum virginicum*)	culver's root	...	...	...	...	...	−	− [14]
Lemon oil/extract	...	−	− [16]	...	...	...	...	...
Lemongrass oil/extract	...	+	− [16]	...	...	− [17]	...	...
Lettuce opium	lettuce (wild)	...	...	+	+	...	...	...
Lettuce (wild)	*Lactuca sativa*	...	...	...	...	...	−	− [26]
Licorice	*Glycyrrhiza glabra*	...	...	+	+	...	...	...
Lily of the valley root	...	...	−	−	...	...	...	...
Linseed oil/extract	...	+	− [16]	...	...	− [17]	...	...
Lippia citriodora	...	...	...	...	...	− [14]	...	...
Lithospermum officinale	...	...	...	...	...	− [14]	...	...
Lithospermum purpureo-coeruleum	...	...	...	...	...	− [14]	...	...
Loasa bipinnata	...	...	...	...	...	− [14]	...	...
Loasa chelidonifolia	...	...	...	...	...	− [14]	...	...
Loasa picta	...	...	...	...	...	− [14]	...	...
Lobelia	*Lobelia inflata*	...	...	−	−	...	...	...
Lobelia inflata	Indian tobacco	...	...	...	...	− [14]	...	...
Logwood (Jamaican)	...	...	...	−	−	...	...	...
Lovage	...	...	...	−	−	...	...	...
Lupulin	hops	...	...	−	−	...	...	...
Mace	*Myristica fragrans*	+	− [13]	−	−	...	−	− [12,18]
Madder	...	...	...	−	−	...	...	...
Mandrake (American)	(? podophyllum)	...	...	−	−	...	...	...

Maple leaf	...	...	...	...	...	...	...	[24]
Marjoram	*Marjorana hortensis*	...	...	−	−	...	...	...
Marjorana hortensis	*Origanum vulgare*	...	...	...	...	− [14]	...	...
Marshmallow	marshmallow herb	...	...	−	−	...	...	...
Marshmallow herb	marshmallow	...	...	−	−	...	...	...
Mate	...	...	...	−	−	...	...	...
Meadowsweet herb	...	...	...	−	−	...	...	...
Melampyrum americanum	...	...	...	...	...	− [14]	...	...
Melilot herb	...	...	...	−	−	...	...	...
Melissa officinalis	balm herb	...	...	...	...	− [15]	...	...
Melochia herb	...	...	...	−	−	...	...	...
Melothria qualalupensis	...	...	...	...	...	− [14]	...	...
Mentha citrata	...	...	...	...	...	− [14]	...	...
Mentha crispa	...	...	...	...	...	− [14]	...	...
Mentha gentilis	...	...	...	...	...	− [14]	...	...
Mentha piperita	peppermint	...	...	...	...	− [14,15]	...	...
Mentha pulegium	pennyroyal	...	...	...	...	+ [14]	+	− [14]
Mentha requientii	...	...	...	...	...	+ [14]	+	− [14]
Mentha rotundifolia	...	...	...	...	...	− [14]	...	...
Mentha spicata	mint	...	...	...	...	− [14]	...	...
Mentzelia albescens	...	...	...	...	...	+ [14]	+	− [14]
Mint	spearmint	...	...	−	−	...	...	...
Molohia (jute)	...	...	...	−	−	...	...	...
Motherwort	...	...	...	−	−	...	−	− [26]
Mountain flax	...	...	...	−	−	...	...	...
Mugwort	...	...	...	−	−	...	...	...
Mullein	*Verbascum thapsus*	...	...	−	−	...	...	...
Mustard (black)	...	...	...	+	−	...	...	...
Mustard dilo oil	...	...	...	...	...	− [17]	...	...
Myosotis sylvatica	...	...	...	...	...	− [14]	...	...
Myriocarpa brachystachys	...	...	...	...	...	− [14]	...	...
Myristica fragrans	nutmeg	...	...	...	...	...	−	− [14]
Myroxylon toluiferum	tolu	...	...	...	...	...	−	− [14]
Myrrhis odorata	gum myrrh	...	...	...	...	...	−	− [14]
Nettle	...	...	...	−	−	...	...	...
Nicotiana tabacum	tobacco	...	...	...	...	− [15]	...	...
Nutmeg	nutmeg oil	+	− [13]	+	+	...	−	− [13,14]
							?+	+ [18]
Nutmeg oil/extract	mace	?+	+ [16]	...	...	− [17]	−	− [18]

TABLE 1—*Continued.*

Test Material	Cross Reference	RD-L A	RD-L B	MD-L [13] A	MD-L [13] B	D-N	D-L A	D-L B
Nux vomica	...	...	...	−	−	...	...	...
Odontites verna	...	...	...	...	...	− [14]	...	...
Olive oil/extract	olive pits (ground)	+	− [16]	...	...	− [17]	...	...
Olive pits (ground)	olive oil/extract	...	...	−	−	...	...	...
Onion	...	...	...	−	−	...	...	...
Opium	*Papaver somniferum*	...	...	−	−	...	...	...
Opopanax	...	...	...	−	−	...	...	...
Orange oil/extract	...	−	− [16]	...	...	...	...	...
Oregano	...	...	...	...	...	...	...	− [24]
Origanum vulgare	marjoram	...	...	...	...	− [14,15]	...	...
Orris	poison flag	...	...	+	+	...	...	...
Palm kernel meal	...	...	...	−	−	...	...	...
Papaver somniferum	poppy petals	...	...	...	...	− [15]	...	...
Parasponia andersonii	...	...	...	...	...	− [15]	...	...
Parietaria officinalis	...	...	...	...	...	− [14]	...	...
Parsley	parsley oil	...	...	−	−	...	...	...
Parsley oil/extract	parsley	−	− [16]	...	...	...	−	− [18]
Passionflower leaf	...	...	...	−	−	...	...	...
Patchouli oil/extract	...	+	+ [16]	...	...	...	+	?+ [18]
Peanut oil/extract	...	+	− [16]	...	...	− [17]	...	...
Pedicularis canadensis	wood betony	...	...	...	...	...	−	− [14]
Pelargonium capitatum	...	...	...	...	...	+ [15]	...	− [14]
Pellitory root	pyrethrum	...	...	−	−	...	...	...
Pennyroyal	pennyroyal oil	...	...	−	−	...	...	...
Pennyroyal oil/extract	*Mentha pulegium*	+	+ [16]	...	...	...	...	...
Pepper (black)	...	...	...	−	−	...	...	...
Pepper (black alligator)	...	...	...	−	−	...	...	...
Peppermint herb	peppermint oil	...	...	−	−	...	...	− [24]
Peppermint oil/extract	*Mentha piperita*	+	+ [16]	...	...	...	+	+ [18]
Peruvian balsam	...	−	− [16]	...	...	− [10]	−	− [18]
Petalonyx thurberi	...	...	...	...	...	− [14]	...	...
Phlomis	...	...	...	−	−	...	...	...

Pilewort	...	...	...	−	−	...	...	...
Pimento	...	...	...	+	−	...	...	...
Pine oil/extract	turpentine oil	+	− [16]	...	...	...	...	...
Podophyllum	mandrake (American) (?)	...	...	−	−	...	...	...
Poison flag	*Iris florentina*	...	...	+	+	...	...	...
Poppy petals	opium	...	...	−	−	...	...	...
Proteum icicariba	gum animi	...	...	...	...	...	−	− [14]
Pulsatilla	...	...	...	−	−	...	...	...
Pyrethrum	Pellitory root	...	...	−	−	...	...	...
Quassia	...	...	...	−	−	...	...	...
Quercitron bark	...	...	...	−	−	...	...	...
Quillaja bark	...	...	...	−	−	...	...	...
Ragweed	...	...	...	...	...	...	...	− [24]
Ragwort	senecio	...	...	−	−	...	...	...
Rapeseed	...	...	...	−	−	...	...	...
Raspberry leaves	...	...	...	−	−	...	...	...
Rauwolfia	...	...	...	−	−	...	...	...
Rhamnus frangula	...	...	...	...	...	+ [15]	...	− [14]
Rhubarb (root)	...	...	...	−	−	...	...	...
Rosmarinus officinalis	rosemary	...	...	...	...	− [14,15]	...	...
Rosemary	rosemary oil	...	...	−	−	...	...	− [24]
Rosemary oil/extract	*Rosmarinus officinalis*	+	− [16]	...	...	− [17]	...	...
Rue	rue oil	...	...	−	−	...	...	...
Rue oil/extract	rue	+	− [16]	...	...	...	...	...
Ruellia occidentalis	...	...	...	...	...	− [14]	...	...
Rupturewort	...	...	...	−	−	...	...	...
Sabadilla	...	...	...	−	−	...	...	...
Sabal	...	...	...	+	−	...	...	...
Safflower	...	...	...	−	−	...	...	...
Saffron	...	...	...	−	−	...	...	...
Sagapenum	...	...	...	+	+	...	...	...
Sage	sage (Dalmatian)	−	− [16]	−	−	...	...	− [24]
Sage (Dalmatian)	*Salvia officinalis*	...	...	...	...	...	+	− [11]
St.-John's-wort	...	...	...	−	−	...	...	...
Salvia divinorum	...	...	...	...	...	− [14]	...	...
Salvia leucantha	...	...	...	...	...	− [14]	...	...
Salvia officinalis	sage	...	...	...	...	+ [15]	...	− [14]
Salvia rutilaus	...	...	...	...	...	− [14]	...	...

TABLE 1—*Continued.*

Test Material	Cross Reference	RD-L		MD-L [13]		D-N	D-L	
		A	B	A	B		A	B
Sandalwood	...	...	...	...	...	...	−	− [18]
Sandalwood oil	...	...	...	...	...	− [17]	+	− [18]
Sandal oil/extract	sandal wood (red)	+	+ [16]	...	...	− [10]	...	...
Sandal wood (red)	sandal wood (yellow)	...	...	+	+	...	...	...
Sandal wood (yellow)	*Adenanthera pavonina*	...	...	−	−	...	...	...
Sanicle herb	...	...	...	−	−	...	...	...
Sarsaparilla	...	...	...	−	−	...	...	...
Satureia hortensis	...	...	...	...	...	+ [15]	...	− [14]
Satureia montana	...	...	...	...	...	− [14]	...	...
Savin	savin oil	...	...	+	−	...	...	...
Savin oil/extract	juniper oil	+	+ [16]	...	...	...	...	...
Savory	...	...	...	−	−	...	...	− [24]
Scammonyroot	...	...	...	−	−	...	...	...
Seaweed (edible)	...	...	...	−	−	...	...	...
Senecio	ragwort	...	...	−	−	...	...	...
Senaga	...	...	...	−	−	...	...	...
Senna	...	...	...	−	−	...	...	...
Sesame oil	...	...	...	...	...	− [17]	...	...
Shepherd's purse	...	...	...	−	−	...	...	...
Silverweed	...	...	...	−	−	...	...	...
Skullcap herb	...	...	...	−	−	...	...	− [24]
Slippery elm bark	...	...	...	+	−	...	...	...
Sloe leaves	...	...	...	−	−	...	...	...
Snakeroot (black)	...	...	...	−	−	...	...	...
Snakeroot (Virginian)	...	...	...	−	−	...	...	...
Southernwood herb	...	...	...	−	−	...	...	...
Soya	...	...	...	+	−	...	...	...
Spearmint	spearmint oil	...	...	−	−	...	...	− [24]
Spearmint oil/extract	*Mentha spicata*	+	− [16]	...	...	...	−	− [18]
Squaw vine (partridgeberry)	...	...	...	+	−	...	...	...
Squill	squill (red)	...	...	−	−	...	...	...

Squill (red)	squill	...	...	+	−	...	...	...
Star grass	...	...	...	−	−	...	...	...
Starwort	...	...	...	−	−	...	...	...
Stavesacre seeds	...	...	...	−	−	...	...	...
Stonecrop herb	...	...	...	−	−	...	...	...
Stramonium	*Datura stramonium*	...	...	−	−	...	...	− [24]
Strobilanthes isopricus	...	...	...	...	...	− [14]	...	...
Strobilanthes lactatus	...	...	...	...	...	+ [14]	+	− [14]
Stropanthus	...	...	...	+	−	...	...	...
Sumac	...	...	...	−	−	...	...	...
Symphytum officinale	comfrey	...	...	...	...	− [14]	...	...
Tansy	...	...	...	−	−	...	...	...
Tarragon	...	...	...	−	−	...	...	...
Tea	tea (instant)	...	...	+	−	...	...	...
Tea (instant)	tea plant	...	...	+	−	...	...	...
Tea plant	tea	...	...	−	−	...	...	...
Terramerium nervosum	...	...	...	...	...	− [14]	...	...
Teucrium scorodonia	wood sage	...	...	...	...	...	+	− [14]
Thuja	*Thuja occidentalis*	...	...	+	+	...	...	...
Thuja occidentalis	thuja	...	...	...	...	...	+	− [14]
Thunbergia alata	...	...	...	...	...	− [14]	...	...
Thyme	*Thymus vulgaris*	−	− [20]	−	−	...	+	− [10,24]
							−	− [18]
Thymus herba-baron	...	...	...	...	...	− [14]	...	...
Thymus serphyllum	...	...	...	...	...	− [14]	...	...
Thymus vulgaris	thyme	...	...	...	...	+ [15]	...	− [14]
Ti tree	...	...	...	...	...	− [17]	...	...
Tobacco	tobacco, cigar leaf	...	...	−	−	...	...	...
Tobacco, cigar leaf	tobacco, flue cured	...	...	...	...	− [26]	...	...
Tobacco, flue cured	tobacco (Turkish)	...	...	...	...	− [26]	...	...
Tobacco (Turkish)	*Nicotiana tabacum*	...	...	...	...	...	+	− [11]
Tolu	*Myroxylon toluiferum*	...	...	+	+	...	...	...
Tournefortia paniculata	...	...	...	...	...	− [14]	...	...
Tournefortia peruviniana	...	...	...	...	...	− [14]	...	...
Tournefortia scabra	...	...	...	...	...	− [14]	...	...
Trema cannabina	...	...	...	...	...	− [14]	...	...
Trema micrantha	...	...	...	...	...	− [14]	...	...
Tung oil	...	...	...	...	...	− [17]	...	...
Turmeric	...	...	...	−	−	...	...	...

TABLE 1—*Continued.*

Test Material	Cross Reference	RD-L A	RD-L B	MD-L [13] A	MD-L [13] B	D-N	D-L A	D-L B
Turpentine oil/extract	colophony	+	− [16]	...	...	− [17]	...	...
Ulmus alata	...	...	...	...	...	+ [14]	+	− [14]
Ulmus campestris	...	...	...	...	...	+ [14]	+	− [14]
Ulmus crassifolia	...	...	...	...	...	− [14]	...	...
Ulmus divaricata	...	...	...	...	...	+ [14]	+	− [14]
Ulmus foliaceae	...	...	...	...	...	+ [14]	+	− [14]
Ulmus pedunculata	...	...	...	...	...	− [14]	...	...
Ulmus rubra	...	...	...	...	...	− [14]	...	...
Ulmus scabra	...	...	...	...	...	− [14]	...	...
Urtica dioica	...	...	...	...	...	− [14]	...	...
Urtica urens	...	...	...	...	...	− [14]	...	...
Valerian root	...	...	...	−	−	...	...	...
Verbascum thapsus	mullein	...	...	...	...	− [26]	...	...
Verbena officianalis	verbena oil	...	...	...	...	− [14]	...	...
Verbena oil	*Verbena officianalis*	...	...	...	...	− [17]	...	...
Vervain	...	...	...	−	−	...	...	...
Vetiver oil	...	...	...	...	...	− [17]	...	...
Wild cherry bark	...	...	...	+	−	...	...	...
Wintergreen	...	...	...	+	−	...	...	...
Wintergreen oil/extract	...	−	− [16]	...	...	...	...	...
Witch hazel leaves	witch hazel oil	...	...	−	−	...	...	...
Witch hazel oil/extract	witch hazel leaves	+	− [16]	...	...	...	...	...
Wood betony	betony	...	...	+	+	...	...	...
Wood sage	*Teucrium scorodonia*	...	...	+	+	...	...	...
Wormwood	*Artemisia* species	...	...	−	−	...	...	...
Yarrow	...	...	...	−	−	...	...	...
Yeast	...	...	...	+	−	...	...	...
Yohimbé	...	...	...	−	−	...	...	...

[a] + and − indicate positive and negative results, respectively, with respect to color formation in the aqueous (A) and chloroform (B) phases; ?+ indicates the result to be doubtfully positive. The referenced article should be consulted for experimental conditions; all MD-L are in Ref *13* and nonreferenced results were obtained in these laboratories. In some cases the original spellings have been standardized.

of results to be made. The Latin names employed by Thornton and Nakamura [*14*] have been retained because they attempted to compare their results from leaf material itself with those reported elsewhere from derived substances, such as *Lactuca sativa* with lettuce opium [*14*]. The equivalence (for example, of lettuce leaves and lettuce opium) may well not be exact and their text shows that the authors were well aware of this. Authors' spelling variations have been ignored. The column of cross-references is intended to assist the reader in comparing entries that are almost certainly related through commonality or similarity of species. All the comparable entries are retrieved by following the cross-references in their (alphabetical) sequence; for example, if one enters the nutmeg series at nutmeg, the references are successively to nutmeg oil, mace, *Myristica fragrans,* and so back to nutmeg. Several papers have described results from various coffees that are not included in Table 1.

All of the species examined by Thornton and Nakamura were not named in their article [*14*] and a United Nations document [*15*] names only 33 of 122 species examined—the 33 that gave some kind of positive response in various cannabis tests. Table 1 reports results from over 400 herbally derived materials that originated from some 270 different plant species.

The specificities of the tests increase with their increasing number of facets. Thus in Table 1 there are 12 false positives out of 47 RD-L entries; 21, including four species of *Ulmus,* of 143 D-N; 25 of 249 MD-L; and 0 of 73 D-L entries. The false positives deserve further comment.

Smith [*16*] found that 12 out of 40 essential oils or extracts of vegetable origin gave a positive result in an RD-L test. Smith's paper omits reference to Blackie's earlier work [*17*] in which 36 essential oils, fixed oils, and resins (Table 1) were examined by the D-N test. None of these 36 gave a positive test and the list includes 3 (clove, nutmeg, and sandalwood[2]) of Smith's 12, indicating that the preliminary extraction indeed enhances the selectivity of the test. Hughes and Warner [*18*] used the complete D-L test and obtained negative results for 8 (anise, camphor, cardamom, caraway, clove, fennel, nutmeg, and sandalwood) of Smith's list. They also observed that the color of the chloroform layers, although reported [*18*] as blue or red-purple for some oils (patchouli and peppermint) "cannot be confused with the purple of authentic marihuana."[3] Pennyroyal and savin oils were not available for study. Both Smith [*16*] and Hughes and Warner [*18*] reported that their oils were easily distinguished from cannabis by thin-layer chromatography (TLC) employing a Fast Blue B spray reagent. Nine species from which the 12 oils are produced were among the 427 examined by Segelman and Segelman [*19*] by the Rutgers identification of marihuana (RIM) test, the exceptions being *Cinnamomum camphora, Mentha pulegium,* and *M. piperita.* The test involves microscopic examination, plant histology, and staining with Fast Blue B. Eight of these nine species, the exception being *Myristica fragrans,* were categorized by them also as negative for cannabis by TLC. *M. fragrans* (nutmeg) was, in fact, like the other 24 "TLC-positive" (a very generous term) plants, easily distinguished from cannabis by the system. It can be concluded that none of these oils (which neither look nor smell like cannabis) could be confused with cannabis by an analyst employing a D-L test and TLC.

The 25 entries for "positive TLC" [*19*] are alkanet root, three species of aloe, nutmeg, mace, white pine bark and rosin (*Pinus strobus* and *P. palustris*), balm of Gilead bud, black walnut bark, buckthorn berry, butternut root, cascara bark, pale catechu, clove, fishberry, kamala, licorice root, guaiac gum, tansy, thyme, summer savory, Chinese rhubarb, male fern, and wild marjoram. Some form of 20 of these appears in Table 1,

[2]The common names "santalwood" and "sandalwood" appear to be completely synonymous. Sandalwood has been used throughout this paper.

[3]V. J. Warner, Jr., Drug Enforcement Administration, Washington, D.C., personal communication, Dec. 1978.

the exceptions being balm of Gilead, black walnut, fishberry, kamala, and male fern. Of these 20, only licorice root gave a positive test in the most selective Duquénois test employed (MD-L for licorice, Table 1). It is reemphasized that none of the 427 species could be confused with cannabis by TLC [*19*].

The possibility of false positives arising in cannabis testing by combined D-L and TLC has been studied with regard to 25 species listed by de Faubert Maunder (quoted in Ref *14*), which are putatively the same as the 25 items that he published as giving false positives in the MD-L [*13*]. Of all the botanical materials examined by de Faubert Maunder [*13*], only agrimony (negative MD-L) and henna (negative RD-L and D-L) gave a red color in his field test with Fast Blue B. This *suggests* that, with the possible exception of these two, all would give negative TLC results (no color development with Fast Blue B). The 25 species do not feature in Segelman and Segelman's list [*19*]. The 25 items published by Maunder [*13*] are calamus, coffee (green), coffee (roasted), Culver's root, ergot, ginger, gum ammoniac, gum animi, gum copal, gum galbanum, gum kauri, gum myrrh, gum sandarac, henna, lettuce opium, licorice, nutmeg, orris, poison flag, sagapenum, sandalwood (red), thuja, tolu, wood betony, and wood sage. Thornton and Nakamura [*14*] list 22 species of the 25 communicated to them by Maunder, 21 of which have some correspondence with 22 of the 25 published items [*13*] (equating Maunder's two coffees). The exception appears to be *Caplafer conjugated* [*sic*], which, with *Hymenaea courbaril,* was equated with gum copal [*14*]. The three items not dealt with are ergot, gum galbanum, and sagapenum. All of the 22 gave negative results on D-L examinations [*14*].

Results with various coffee preparations submitted to different types of Duquénois test show that some brands, especially instant types, give false positives by RD-L [*20*] and MD-L tests [*13,21*], and even by the D-L test [*18*]. However, these materials are easily distinguished from cannabis by TLC and spraying with Fast Blue B [*18*], and instant coffee does not physically resemble herbal cannabis.

There is no published report of an obviously botanical material apart from cannabis that gives a positive D-L test. Also, all the indications are that cannabis would be distinguishable from other botanical materials by TLC.

Duquénois Tests Applied to Chemical Substances

The herbal materials discussed above are complex mixtures of individual chemical substances. The results of color testing with single chemical entities were therefore studied separately. Several examples presented in Tables 2 to 7 show that the specificity of Duquénois tests is enhanced by preliminary extraction with petroleum ether or hexane.

Many of the results were obtained by the following RD-L method: a small quantity of test material was placed into a test tube—a clearly visible speck in the case of solids and a tiny but visible smear in the case of oils. These quantities rapidly (30 s) gave satisfactory, intense purple colors when cannabidiol and cannabinol (solids) and tetrahydrocannabinols and cannabis resin (oils) were tested. A few drops of fresh Duquénois reagent were added, the substrate was quickly dissolved by agitation, and an equal volume of concentrated hydrochloric acid was added. Color development was noted. After 3 or 4 min an equal volume of chloroform was added and the partition of colors between the aqueous and organic phases noted. Gauging the volumes of reagents by eye was satisfactory, and if the chloroform and aqueous layers did not separate a drop or two of water was added.

With a quite unreasonable overload of a few steroids and terpenes (several crystals or a drop or two probably totalling more than 1 mg), purplish colors slowly resulted during the RD-L test. This effect was observed with some terpenes by Thornton and Nakamura [*14*]. It is unrealistic to suppose that an experienced analyst could confuse these false positives with cannabis or cannabinoids.

TABLE 2—*Results obtained with terpenes in the Duquénois test.* [a]

	RD-L		D-L [18]	
	A	B	A	B
Abietic acid	− [17]	...	...	...
Borneol	− [27]	...	...	...
Cadinene	− [17]	...	...	...
Camphene	− [17]	...	...	...
Camphor	− [17]	...	− [D-N; 10]	...
Carotene	− [17]	...	...	...
Carvene	− [17]	...	...	...
Caryophyllene	?+ [15]	...	−	−
	?+	− [14]	...	...
Cedrene	− [17]	...	...	...
Cedrol	?+	−	...	...
Cineole	?+ [15]	...	−	−
	?+	− [14]	...	...
Citral	+ [27]	...	−	−
			− [D-N; 10]	
Citral dimethylacetal	...	...	−	−
Citronellal	?+ [27]	...	...	...
	− [17]	...	...	...
	?+	− [14]	−	?+
Citronellol	+ [27]	...	...	...
	− [17]	...	...	...
	+	− [14]	?+	−
p-Cymene	− [15]	− [14]	...	...
Farnesol	+ [27]	...	−	−
Geraniol	+ [27]	...	−	−
	+	− [14]	...	...
	−	−	...	...
Limonene	− [27]	...	− [D-N; 9]	...
	+	− [14]	...	...
Linalool	+ [27]	...	−	−
	?+	− [14]	...	...
	?+	−	...	...
p-Menthadienol	−	−	...	...
Menthol	− [15]	...	...	...
	−	− [14]	...	...
Myrcene	?+	− [14]	...	...
Nerol	+ [27]	...	−	−
	+	− [14]	...	...
α-phellandrene	+ [27]	...	−	−
	+	− [14]	...	...
Phytosterol	− [17]	...	...	...
Pinene	− [27]	...	?+	−
	− [17]	...	...	...
	−	− [14]	...	...
Pulegone	− [27]	...	?+	?+
	+	+ [14]	...	...
	−	−	...	...
Squalene	− [27]	...	...	...
Terpineol	− [27]	...	−	−
Thujone	−	− [14]	...	...
Vetivene	− [17]	...	...	...

[a] See footnote, Table 1.

TABLE 3—*Results obtained with miscellaneous phenols and derivatives in the Duquénois test.* [a]

		RD-L		D-L [18]	
	D-N	A	B	A	B
Anethole	...	− [27]	...	...	...
Anisole	...	− [27]	...	...	...
8-Benzoflavone	...	...	...	−	−
Butylated hydroxytoluene (BHT)	...	?+	−	...	...
Carvacrol	...	− [27]	...	...	...
	...	+	− [14]	?+	?+
Catechin	...	...	...	?+	−
Catechol	...	−	− [14]	...	...
	...	−	− [22]	...	...
Cosotoxin	− [9]	...	...	...	...
Cotoin	− [9]	...	...	...	...
Coumarin	− [10]	−	−	...	...
3,4-Dihydroxybenzaldehyde	...	−	−	...	...
4,4-Dihydroxystilbene	...	...	...	−	−
2,6-Dihydroxytoluene	...	?+	− [22]	−	−
3,5-Diiodosalicylic acid	...	−	−	...	...
2,6-Dimethylphenol	...	+	−	...	...
3,4-Dimethoxypropenylbenzene	...	...	...	−	−
2,2-Dimethylchroman-5-ol	...	+	+ [22]	...	...
2,2-Dimethylchroman-7-ol	...	+	+ [22]	...	...
Equilenin	...	−	−	...	...
Equol	...	?+	−	...	...
Estradiol	...	−	−	...	...
Eugenol	...	− [15]	...	?+	?+
	...	−	− [14]	...	...
o-Eugenol	...	...	...	?+	?+
Filicin	− [9]	...	...	...	...
Flavone	− [10]	...	...	−	−
Guaiacol	− [10]	...	...	...	...
Hexylresorcinol	...	+	− [14]	+	−
	...	?+	− [22]	...	...
Hydroquinone	...	−	− [14]	...	...
	...	−	− [22]	...	...
2-Hydroxy-4-methylbenzoic acid	...	−	−	...	...
2-Hydroxy-3-methoxy-5-methyl allylbenzene	...	−	−	...	...
2-hydroxy-4-methoxy-propenylbenzene	...	+	+	...	...
Isoeugenol	− [10]	...	...	?+	−
Isouegenol methyl ether	...	− [27]	...	...	...
Isosafrole	...	− [27]	...	...	...
Juglone	...	− [27]	...	...	...
4-Methylumbelliferone	...	...	...	−	−
α-Naphthoflavone	...	...	...	−	−
Naphthoresorcinol	...	+	+ [14]	?+	?+
p-Nitrophenol	− [10]	...	...	...	...
Phenol	− [10]	−	− [22]	...	...
	...	−	−	...	...
p-Phenylphenol	...	−	− [14]	...	...
Phloroglucinol	− [9]	+ [15]	...	−	−
	...	+	− [14]	...	...
	...	−	− [22]	...	...
	...	−	−	...	...
Pterocarpine	...	?+	...	...	...
Rottlerine	− [9]	...	...	...	...
Safrole	...	− [27]	...	...	...
Salicylaldehyde	− [10]	...	...	...	...
Salicin	...	− [17]	...	...	...
Salicylic acid	− [10]	...	...	...	...
p-Styrylphenol	...	...	...	−	−

TABLE 3—*Continued*

	D-N	RD-L A	RD-L B	D-L [18] A	D-L [18] B
Thymol	...	+ [15]	...	−	−
	...	+ [27]	...	...	...
	...	?+	− [14]	...	...
	...	?+	−	...	...
α-Tocopherol	...	...	...	−	−
2,3,4-Trihydroxybenzoic acid	...	−	−	...	...
2,2,5-Trimethylchroman-7-ol	...	+	+ [22]	...	...
2,2,7-Trimethylchroman-5-ol	...	+	+ [22]	...	...
2,2,7-Trimethylchroman-5-ol-4-one	...	− [22]	...	...	...

[a] See footnote, Table 1.

TABLE 4—*Results obtained with 1,3-dihydroxybenzenes in the RD-L test.* [a]

	A	B		A	B
5-H (resorcinol) [b]	−	−	5-C_6H_{13}	+	+
5-CH_3 (orcinol) [c]	−	−	5-C_7H_{15}	+	+
5-C_2H_5	+	+	5-$CH_2C_6H_5$	+	+
5-C_3H_7	+	+	5-CO_2H	−	−
5-C_4H_9	+	+	5-CO_2CH_3	−	−
5-C_5H_{11} (olivetol) [d]	+	+	5-COC_4H_9	−	−
4-C_6H_{13} *see* hexylresorcinol, Table 3					
2-CH_3 *see* 2,6-dihydroxytoluene, Table 3					
5-OH *see* phloroglucinol, Table 3					

[a] This laboratory. See footnote, Table 1.
[b] Also reported are RD-L A: ?+ [27]; RD-L A: ?+ and B: − [22]; D-L A: ?+ and B: − [18].
[c] Also reported are RD-L A: + and B: + [14]; RD-L A: ?+ and B: − [22]; D-L A: + and B: − [18]. The color is similar to but distinguishable from that resulting from cannabis.
[d] Also reported are RD-L A: + and B: + [14]; D-L A: + and B: + [18].

TABLE 5—*Results obtained with 1,3-dimethoxybenzenes in the RD-L test.* [a]

	A	B		A	B
2-CH_3	−	−	2-CO_2CH_3	−	−
5-C_5H_{11}	?+	−	5-CO_2CH_3	−	−
2-CHO	−	−	5-COC_4H_9	−	−
2-CH_2OH	?+	?+	5-CH_2CONH_2	?+	−
5-CO_2H	−	−			

[a] This laboratory; see footnote, Table 1.

Since terpenes are ubiquitous in nature, they were among the earliest of compounds on which the specificity of the Duquénois test was investigated. Results for 32 terpenes are compiled in Table 2. There appears to be a very doubtful possibility that citronellal and pulegone give a false positive in the chloroform layer of the D-L test. Both are easily distinguished from cannabis resin by TLC [18]. Results obtained with miscellaneous phenols and derivatives (Table 3) stongly support the deduction [22] that 1,3-dioxy substitution (resorcinol substitution) on a benzene ring is important for color development. Results obtained with 1,3-dihydroxybenzenes (simple resorcinol derivatives) are therefore separately presented in Table 4. It can be seen that alkyl substitution at C-5 greater than

TABLE 6—*Results obtained with cannabinoids in the Duquénois test.* [a]

	RD-L		D-L [18]	
	A	B	A	B
Cannabichromene	+	+	+	+
Cannabidiol	+ [15]	...	+	+
	+	+ [14,22]	...	...
	+	+	...	...
Cannabidiol dimethyl ether	+	+ [22]	...	...
Cannabidiolic acid	+	+ [14]	...	...
Cannabigerol	+	+	+	+
Cannabinol	+	+ [14,22]	+	+
	+	+	...	...
Cannabinolic acid	...	...	+	+
Cannabinol acetate	...	...	+	+
Hexahydrocannabinol methyl ether-9-chloro	+	+	...	...
Pyrahexyl	+	+ [14]	...	...
Tetrahydrocannabidiol	+	+ [22]	...	...
Δ^8-THC	+	+ [14,22]	+	+
	+	+	...	...
Δ^8-THC benzylether	?+	?+ [22]	...	...
Δ^8-THC-2-bromo	−	−	...	...
Δ^8-THC-2,4-dibromo	−	−	...	...
Δ^8-THC methylether	+	+	...	...
Δ^8-THC methylether-4-bromo	−	−	...	...
Δ^8-THC-2-tetraacetylglucoside	+	−	...	...
Δ^9-THC	+	+ [14,22]	+	+
	+	+	...	...
Δ^9-THC acetate	?+	+ [14]	...	...
Δ^9-THC "unnatural"	+	+	...	...

[a] See footnote, Table 1.

methyl leads to positive results. Purple colors did not develop when a carbonyl group was substituted at C-5.

Results with 1,3-dimethoxybenzenes are reported in Table 5. It is interesting to note that the color from olivetol dimethyl ether was blue and the color extracted into chloroform was light blue, fairly easily distinguished when directly compared with the colors from cannabis. Results obtained with cannabinoids are reported in Table 6. Of the 21 compounds listed, three derivatives of Δ^8-THC, 2-bromo-, 2,4-dibromo-, and 4-bromomethyl ether, gave negative results. The results suggest that the highly reactive C-2 position is involved in production of the purple entity and that when it is blocked by substitution or by steric crowding, the Duquénois reaction is prevented or hindered. Cannabinoids are the predominant group of compounds found in cannabis having the 1,3-dioxybenzene substitution, and the development of the color can be ascribed to their presence. Table 7 reports results from miscellaneous materials. Carbohydrates failed to develop colors with the reagent, and only 3,5-dimethoxyphenylacetamide of 33 nitrogenous materials gave a positive result. This result was reported in Table 5; the color was considered to be pale turquoise, readily distinguishable from cannabis.

Of course, the compounds listed in Tables 2 to 7 are but a drop in the bucket of organic substances that could be tested. Hundreds of substances are commonly available in chemical stores. The results nevertheless suggest there is a limited number of predominantly research chemicals that could give false positives in the Duquénois test. Simple TLC would distinguish these single chemical substances from cannabis preparations, which contain several readily detected, characteristic components. It is hoped that continued

TABLE 7—*Results obtained with miscellaneous materials in the Duquénois test.* [a]

The following gave negative results in the aqueous phase, RD-L test:

Ascorbic acid, dextrose, galactose, lactose, levulose, mannose, quebrachitol [*14*]
Sucrose [*17*]
β-D-Glucose and its penta-acetate gave negative results in A and B, RD-L test
Cinnamic aldehyde [*27*]
Ethyl hydnocarpate [*17*]
Guaiazulene [*27*]
Meconic acid [*17*]
Note: guaiazulene was considered to give positive results in a time-extended D-L test [*18*] and furfural gave a doubtful positive in A, RD-L test [*15*]
Alkaloids: atropine, aconitine, berberine, brucine, caffeine, cinchonine, cocaine, codeine, colchicum, ecgonine, homatropine, hydrastine, morphine, narceine, narcotine, nicotine, nicotinic acid, papaverine, strychnine, theobromine [*17*]
Other nitrogenous substances: 4-hydroxy-3-methoxy-β-nitrostyrene and the following β-methyl derivatives: 3,4-$(OH)_2$, 3-OH-4-OCH_3, 4-OH-3-OCH_3, 4-OH-3-OC_2H_5, 4-$N(CH_3)_2$
Barbitone [*17*]
5-Chloro-2-hydroxyaniline; phenyltoloxamine; pyrrole [*27*]
Styphnic acid [*14*]
1′3′3′-Trimethyl-6-hydroxyspiro-2*H*-1-benzopyran-2,2′-indoline D-L A, − and B, − [*18*].

Duquénois and Negm [*10*] reported negative results in the aqueous phase for several of the foregoing and for the following:

Acetanilide, acetophenetidin (phenacetin), acetylsalicylic acid (aspirin), aminoacetophenone, aminopyrine, ammonium succinate, amylocaine, antipyrine, aspartic acid, cevadine (veratrine), cholesterol, cinnamic acid, citric acid, dimethylaminoazobenzene, dimethylaniline, dinitrobenzene, hydroxylamine, indole, isatin, lactic acid, malic acid, mandelic acid, meconic acid, methenamine, β-napthoquinone, nitronaphthol, nitrosonaphthol, *m*-phenylenediamine, phenylhydrazine, picrotoxin, piperine, procaine, quinic acid, quinine, quinone, saccharin, succinic acid, sulfanilic acid, sulfonmethane, theophylline, urea, uric acid, xanthydrol, and less well-defined materials (for example, "albumins" and "aliphatic acids").

[a] See footnote, Table 1.

testing with model compounds and extension to cannabinoids will lead to the delineation of the Duquénois reaction. An interesting result reported in Table 3 is that from equol, since the paper by Pitt et al [*22*] implies that equol would give a positive Duquenois test. Pure equol turns out to be a rare commodity. With the reagent, equol rather slowly developed a blue color, which was almost completely discharged on addition of the chloroform. After further standing for about 10 min, the aqueous layer was faintly blue but the chloroform layer remained colorless.

Conclusion

The D-L test for cannabis relies on the presence of cannabinoids, which produce chloroform-soluble purple colors on reaction with the reagent. Coupled with a simple inspection for gross appearance and odor and microscopic examination, it continues to be used for the identification of samples of hashish and marihuana. The additional use of TLC further ensures the correctness of the identification and is advisable for all herbal samples since it appears to be more discriminatory than the D-L test. The test alone should not be used for preparations that have no botanical characteristics, such as liquid hashish; additional tests such as TLC in comparison with authenticated cannabis must be carried out. Since the D-L test is very simple and rapid, it should be applied to suspected cannabis samples; unless special circumstances dictate otherwise, negative results indicate that testing for cannabis need proceed no further.

Subsequent to the preparation of this manuscript, experimental work has been published that confirms the suitability of the D-L test combined with microscopic examination and TLC for the identification of cannabis [*23*].

Summary

The Duquénois test is employed throughout the world as part of the identification procedure for cannabis. A survey of results published for more than 400 herbally derived and botanical materials originating from some 270 different plant species is presented. A survey of results from some 200 organic compounds is also presented. The data show that the D-L modification is the most specific. There is no published report of an obviously botanical material, apart from cannabis, that gives a positive D-L test. Apart from cannabinoids, some resorcinol derivatives give a positive test. All of the materials are easily distinguished from cannabis by TLC.

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