Constituents of *Cannabis sativa* L. XI: Cannabidiol and Cannabichromene in Samples of Known Geographical Origin

Keyphrases ☐ *Cannabis sativa* L.—constituents, cannabidiol and cannabichromene in samples of known geographical origin ☐ Cannabidiol—analysis, samples of known geographical origin ☐ Cannabichromene—analysis, samples of known geographical origin ☐ Marijuana—constituents of *Cannabis sativa*, samples of known geographical origin

To the Editor:

Recent development of simplified methods for the separation of cannabidiol (I) and cannabichromene (II) as well as reports supporting the interactions between cannabinoids prompted this communication. We wish to report the presence of I and II in freshly grown *Cannabis* from known geographical origin.

The separation of pure I and II by conventional GLC techniques was reported (1), but this separation was insufficient for quantitation. Turner and Hadley (2) obtained a clear and discrete separation of I and II by the use of trimethylsilyl ether derivatives. Turner *et al.* (3) then described the use of silyl derivatives in routine analysis, which provided a method for quantitating I and II.

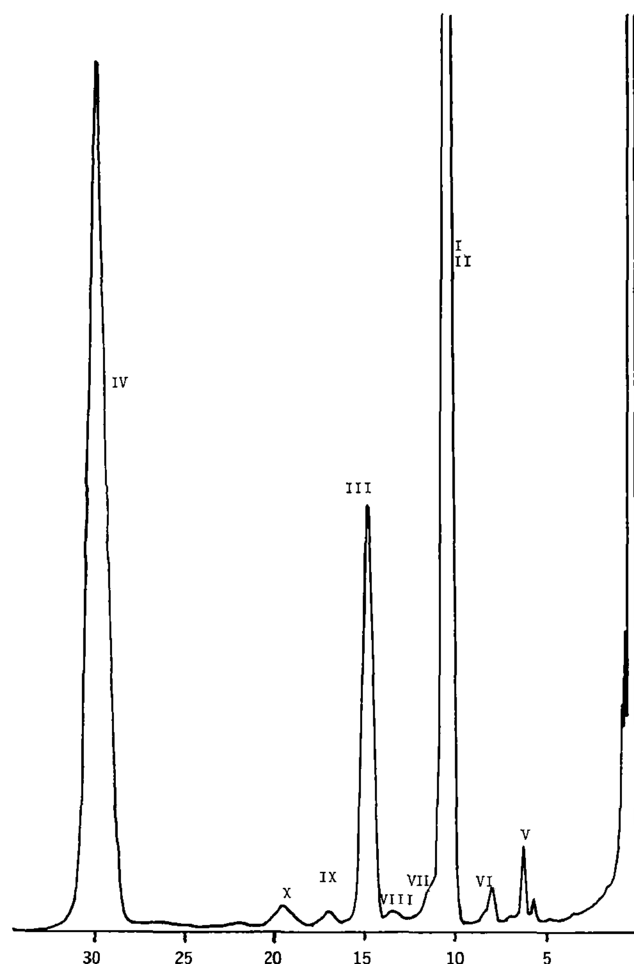

Figure 1—*Chromatogram of the Moroccan variant, MO-A male, showing: I, cannabidiol; II, cannabichromene; III,* (−)-Δ^9-trans-*tetrahydrocannabinol; X, cannabinol; and IV, androst-4-ene-3,17-dione, the internal standard. Also shown are: V, cannabidivarin; VI, combined cannabicyclol and* (−)-Δ^9-trans-*tetrahydrocannabivarin; VII, cannabigerol monomethyl ether; VIII,* (−)-Δ^8-trans-*tetrahydrocannabinol; and IX, cannabigerol.*

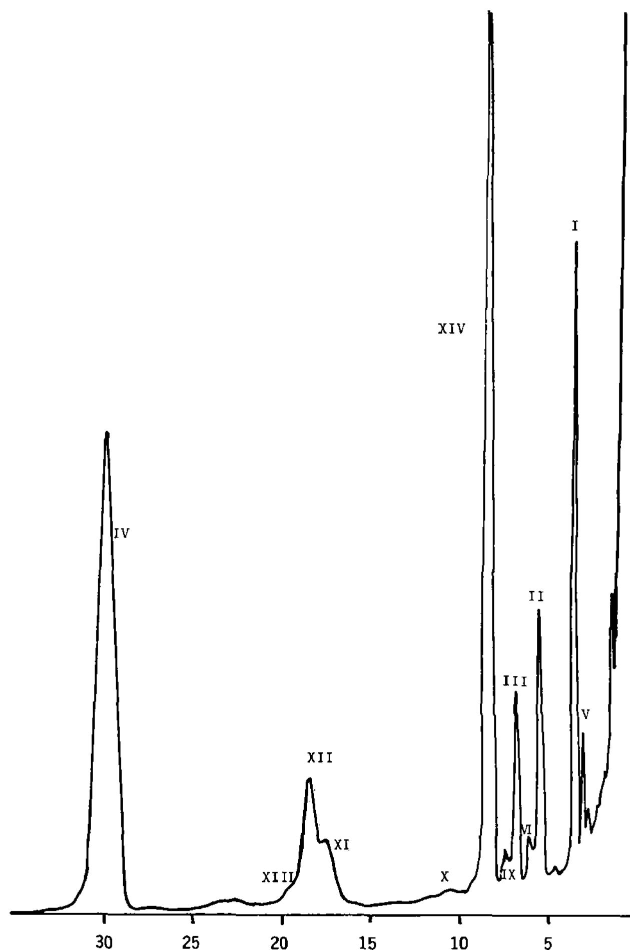

Figure 2—*Chromatogram of the silylated plant material MO-A, Moroccan male variant, showing the first method for the separation of cannabidiol (I) and cannabichromene (II). Also shown are: III,* (−)-Δ^9-trans-*tetrahydrocannabinol; IV, androst-4-ene-3,17-dione, the internal standard; V, cannabidivarin monosilylated; VI, cannabicyclol; IX, cannabigerol disilylated; XIV, cannabidiolic acid; X, cannabinol; XI, unknown; XII,* (−)-Δ^9-trans-*tetrahydrocannabinolic acid A; and XIII,* (−)-Δ^9-trans-*tetrahydrocannabinolic acid B.*

As early as 1970, Carlini *et al.* (4) observed that I could possibly block some effects of (−)-Δ^9-*trans*-tetrahydrocannabinol (III). More recently, I was reported to block several effects of III in animal models and to potentiate the analgesic effects of cannabinol (5). Cannabidiol induced reduction of the hypothermic response to III in rats and rabbits (6).

De Zeeuw *et al.* (7) reported that propyl cannabinoids seem to be more abundant in *Cannabis* grown in India, Nepal, and Pakistan. Turner and Hadley (8), using only samples of known geographical locations, confirmed and extended the previously thought abundance of propyl cannabinoids. Following this example, we investigated many variants of *Cannabis* from known geographical origins as to

Table I—Variants Grown in Mississippi

Geographical Origin	Seed Code	Sex[a]	I[b]	II	III
Afghanistan	AF-B	M	1.94	0.50	2.68
	AF-B	F	1.26	0.01	0.59
	AF-B(1)/C-71	F	0.19	0.83	2.11
	AF-B(1)/C-71	M	4.58	0.27	2.63
	AF-A(1)/C-71	M	0.11	0.77	4.41
	AF-C	Y	0.03	0.11	2.34
Brazil	BR-A	F	t	0.03	2.16
Canary Islands	CI-A	M	1.48	0.03	0.84
Czechoslovakia	CZ-A	F	1.22	0.04	1.11
	CZ-A	M	1.08	0.08	0.54
	CZ-B	F	0.29	0.02	1.03
	CZ-B	M	1.28	0.65	0.08
Ethiopia	ET-A	M	3.05	0.15	1.29
Fibriman	FI-A	F	0.94	0	0.05
	FI-A	M	1.12	0.18	0.06
France	FR-B	F	0.95	0.13	1.49
	FR-C	F	0.11	0.38	3.20
	FR-F	F	1.36	0	0.07
	FR-F	M	0.48	0.24	0.38
	FR-G	F	0.90	0	0.06
	FR-G	M	1.07	0	0.06
	FR-H	M	1.35	0.84	0.08
Ghana	GH-A	Y	0.01	0.08	1.68
	GH-B	Y	t	0.13	2.10
	GH-C	Y	0.02	0.09	2.60
India	IN-A	F	0.03	0.16	1.78
	IN-A	M	0.02	0.10	4.30
	IN-A	Y	0.01	0.15	0.72
	IN-A(1)/C-70	F	0.02	0.10	2.21
	IN-A(1)/C-70	M	0.3	0.20	1.70
	IN-A(2)/C-71	F	0.10	1.72	0.86
	IN-B(2)/C-71	F	0.02	0.26	2.72
	IN-B(2)/C-71	M	2.24	0	0.11
	IN-B(3)/C-72	F	t	0.64	1.95
	IN-B(3)/C-72	M	0	0.47	1.39
	IN-D	Y	0.01	0.40	1.68
	IN-E	F	0.02	0.52	3.31
	IN-F	M	0.03	1.36	0.72
	IN-F	Y	0.08	0.83	3.37
	IN-I	Y	3.40	0.26	0.99
Iowa	IO-A	F	1.70	0.02	0.10
	IO-A	M	1.66	0.04	0.09
	IO-A	Y	t	0.20	1.68
Iran	IR-A	Y	0.91	0	0.06
	IR-A(1)/C-71	F	0.73	0	0.72
	IR-A(1)/C-71	M	t	0.07	1.40
	IR-A(2)/C-72	F	0.07	0.01	0.33
	IR-A(2)/C-72	M	1.63	0.03	0.18
Jamaica	JA-A	Y	t	0.22	1.84
	JA-B	Y	0.01	0.41	2.20
	JA-C	Y	0.81	0.01	0.04
Kenya	KE-A	M	0.01	0.07	1.84
Korea	KO-A	Y	0.03	0.13	1.17
	KO-A(1)/C-70	Y	0.36	0.17	1.39
	KO-A(2)/C-71	M	0.02	0.91	3.83
	KO-A(2)/C-71	Y	t	0.09	0.34
	KO-B(1)/C-70	M	0.01	0.23	0.94
	KO-B(1)/C-70	Y	t	0.11	0.62
Lebanon	LE-A(1)/C-71	F	1.68	0.05	1.07
Manchuria	MN-A	F	1.93	0.23	1.99
	MN-A	M	0	0.56	1.48
Morocco	MO-A(1)/C-70	F	1.61	0	0.08
	MO-A(1)/C-70	M	0.95	0.38	0.39
	MO-B	F	1.84	0.02	0.54
	MO-C	F	0.70	0.11	0.71
	MO-C	M	0.37	0.08	0.16
Nepal	NE-C	Y	0.02	0.28	2.75
Nigeria	NI-D	Y	0.02	0.09	1.86
Pakistan	PK-A	F	t	0.05	1.32
	PK-A	M	0.40	0.19	1.51
	PK-A(1)/C-71	F	1.27	0.23	0.71
	PK-A(1)/C-71	M	1.20	0.04	1.37
Peru	PU-A	M	0.49	0.07	0.04
	PU-A	Y	t	0.08	2.06
Poland	PO-A	F	1.09	0.01	0.06
	PO-A	M	0.69	0.28	0.04
Russia	RU-A(1)/C-70	F	1.79	0.03	0.10
	RU-A(1)/C-70	M	0.04	0.21	1.05
Senegal	SE-A	Y	0.06	0.20	3.55
Sierra Leone	SL-A	F	0.02	0.03	1.23
South Africa	SA-A	X	0	0.11	1.60
	SA-A(1)/C-70	X	0	0.27	1.18
	SA-A(1)/C-71	F	0.03	0.61	3.02
	SA-A(2)/C-71	F	0.55	0.01	2.00
	SA-A(2)/C-71	M	0.05	0.68	2.89
	SA-A(2)/C-71	Y	0	0.06	0.84
	SA-D	F	t	0.15	1.84
	SA-D	Y	0.02	0.40	6.09
	SA-E	F	0.06	0.01	0.63
	SA-F	F	0.01	0.01	0.33
Sudan	SU-A	F	0.03	0.08	2.10
Thailand	TI-B(1)/C-Mon-70	F	0.02	0.42	2.91
	TI-B(1)/C-Mon-70	M	2.27	0.31	0.93
	TI-C(1)/C-Mon-70	F	2.40	0.06	1.36
	TI-C(1)/C-Mon-70	M	2.20	0.14	1.91
	TI-D(1)/C-Mon-70	F	1.25	0.11	1.56
	TI-D(1)/C-Mon-70	M	0.01	0.05	1.68
	TI-F	Y	0.87	t	0.33
	TI-G	Y	0.06	t	1.33
Turkey	TU-A	F	1.28	0.03	0.05
	TU-A	M	1.62	0.08	0.07
	TU-A(1)/C-68	F	1.91	0.04	2.79
	TU-A(2)/C-69	F	0.78	0.02	0.42
	TU-A(2)/C-69	M	2.79	0.16	1.59
	TU-A(2)/C-71	F	2.22	0.03	0.10
	TU-A(2)/C-71	M	1.87	0.24	0.84
	TU-A(3)/C-H-70	F	1.32	0.01	0.92
	TU-A(3)/C-H-70	M	2.02	0.14	0.09
	TU-C	Y	1.17	0.03	0.07
Viet Nam	VN-A(1)/C-71	M	0.54	0.23	3.23
	VN-A(1)/C-71	Y	t	0.04	0.99
	VN-B	Y	0.02	0.14	4.02

[a] F = female, M = male, Y = young plant, and X = mixture of male and female. [b] Data are reported as percent by dry weight in all tables. t = trace amounts (less than 0.01% by dry weight).

their abundance of I and II in freshly grown material.

Figure 1 shows the routine chromatogram used in determining the concentration of each of the cannabinoids. Here I and II are combined and are under one peak. The same Moroccan variant is shown in Fig. 2 after silylation (2), one method for the separation of I and II. However, with this method, four peaks must be taken into consideration: I monosilylated, I disilylated, cannabidiolic acid, and II. Silylated cannabichromenic acid has yet to be identified. Figure 3 again shows the Moroccan variant as produced by the 6% methyl silicone column described previously (3, 9) and shows excellent separation of I and II.

Data were obtained by a GLC computer[1] analysis based on relative retention times of routine *Cannabis* analyses and synthetic standards (Tables I–III). Table I consists of variants grown in Mississippi, and the variants included in Table II were grown in the listed countries. The data are presented as percentages by dry weight in all tables.

Analyses of Mexican material, coded ME-A[2], grown in Mississippi since 1968, are in Table III. The original ME-A seed was from Acapulco, Guerrero,

[1] Digital PDP-8 computer, interfaced with Beckman GC-45, 72-5, and 65.
[2] Used to supply the National Institute on Drug Abuse with research material.

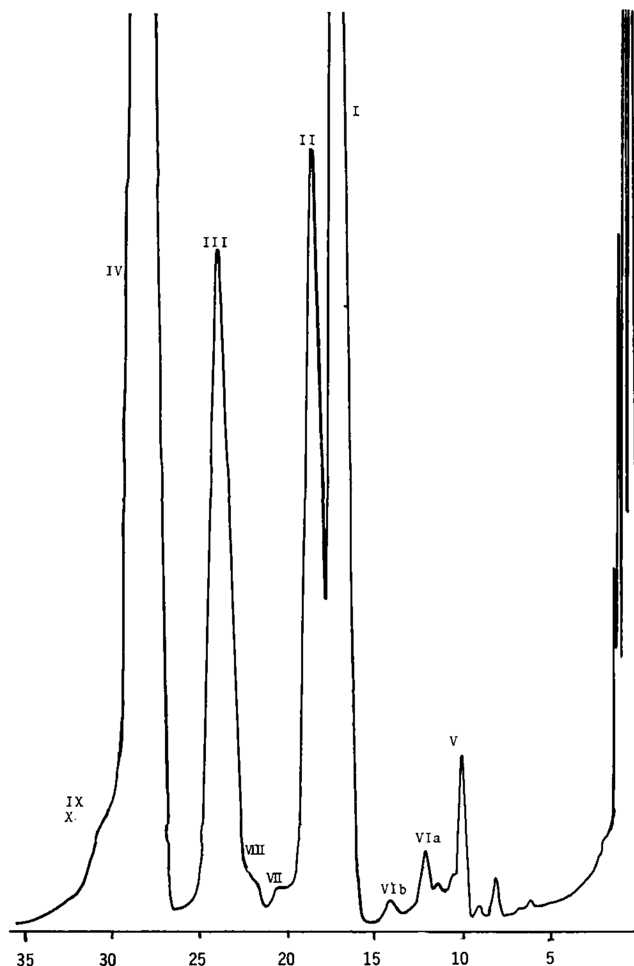

Figure 3—*The Moroccan variant, MO-A male, is shown on the 6% methyl silicone column. Cannabidiol (I) and cannabichromene (II) are adequately separated.* (−)-Δ⁹-trans-*Tetrahydrocannabinol (III), and the internal standard, androst-4-ene-3,17-dione (IV), are illustrated as well as: V, cannabidivarin; VI*a, (−)-Δ⁹-trans-*tetrahydrocannabivarin; VI*b, *cannabicyclol; VII, cannabigerol monomethyl ether; and VIII,* (−)-Δ⁸-trans-*tetrahydrocannabinol. Cannabigerol (IX) and cannabinol (X) are under the internal standard peak.*

Mexico, and analyses of the original plant material showed no I present. However, the fact that Mexican materials produced in our gardens do contain some I indicates that environmental factors affect the ratio of I to II or that some cross-pollination has occurred. The content of III has remained relatively constant when materials were harvested at the same age and time of day (10). A cyclic cannabinoid profile is observed in all variants with regard to age and hour collected; therefore, analyses reported are only for a particular sampling at a particular age and time of day (10).

As previously reported (8, 11), cannabinoid contents vary within each variant in regard to geographical origin and age. Therefore, the data in Tables I and II that show marked variation within a specific geographical location as well as geographical origin in general would be anticipated. Moreover, the presence of II and the absence of I in variants reputed to produce potent marijuana (Costa Rican and Mexican) seem to indicate that some interaction is occurring

Table II—Variants Grown in Their Native Countries

Geographical Origin	Sample	I	II	III
Costa Rica	M-173	0	0.24	3.72
	M-174	0	0.09	1.60
	M-175	0	0.13	1.78
	M-176	0	0.16	1.87
	M-177	0	0.17	1.67
	M-236	0	0.20	1.19
	M-237	0	0.11	1.33
	M-238	0	0.14	2.04
	M-239	0.01	0.20	1.37
	M-240	0	0.17	1.01
Mexico	K24--1A	0	0.09	0.34
	K24--2B	0	0.10	0.14
	K24--3C	0.54	0.09	1.99
	K24--4D	0	0.07	0.59
	K24--5E	0	0.27	2.15
	K24--6F	0	0.15	2.65
	K24--7G	0	0.12	1.00
	K24--8H	0	0.13	1.30
	K24--9I	0	0.16	2.11
	K24--10J	0	0.16	2.97
	K24--11K	0	0.26	2.45
	K24--12L	0	0.24	1.15
	K24--13M	0	0.09	2.66

Table III—Mexican Variants Grown in Mississippi

Seed Code	Sex	I	II	III
ME-A	M	0.06	0.09	1.68
ME-A(1)/C-68	F	0.01	0.10	1.83
ME-A(2)/C-69	M	0.03	0.11	1.11
ME-A(2)/C-69	Y	0.01	0.10	1.35
ME-A(3)/C-71	Y	0.08	0.03	0.53
ME-A(3)/C-71	F	0.16	0.10	1.19
ME-A(2)/C-69	M	0.16	0.01	0.96
ME-A(2)/C-69	F	0.02	0.17	1.74

between II and III. If I antagonizes the action of III (4–6), it is highly possible that II may potentiate the action of HI. This area needs investigating using variants of varying amounts of I to III, II to III, and a mixture of I and II to III. However, it is now clear that II is more abundant in some variants than I and that II can no longer arbitrarily be considered a minor cannabinoid.

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Improved Method for Sampling Hepatic Venous Blood in the Rat

Keyphrases □ Blood sampling, hepatic venous—improved method, rats □ Hepatic blood samples, venous—improved method for sampling in the rat □ Elimination—method for sampling mixed hepatic venous blood proposed, rats

To the Editor:

In an attempt to study hepatic elimination of drugs *in vivo* in the rat, a method for sampling the mixed hepatic venous blood was devised. For estimation of hepatic extraction of drugs *in vivo,* simultaneous measurements of drug concentrations in hepatic arterial, portal venous, and hepatic venous blood are necessary. Extraction of propranolol by the dog liver was studied by direct measurement of hepatic venous concentrations of the drug *in vivo* (1). To obtain hepatic venous blood of the dog, a cannula was introduced into the right or left hepatic vein of the dog *via* a superficial jugular vein under fluoroscopic visualization. This cannulation applied in the dog is not practical for a hepatic vein of the rat, since hepatic veins of the rat are quite narrow and fragile and the cannulation is liable to cause trauma to the liver.

In this communication, we report a simple method for sampling the mixed blood draining from the hepatic veins of the rat in an *in vivo* study of hepatic drug binding and the subsequent drug metabolism. The method described here consists of introducing a cannula (Cannula A, Fig. 1) into the inferior vena cava *via* the external jugular vein so that the mixed blood from the hepatic veins may flow through an artificial channel in the inferior vena cava. Subsequent insertion of another cannula (Cannula B, Fig. 1) into the inferior vena cava makes it possible to sample the mixed hepatic venous blood.

A rat, 270–330 g, is placed in supine position and is anesthetized lightly with ether at suitable intervals. The abdomen is opened through a midline incision extending from the symphysis pubis to the xiphoid. The right lobe of the liver is reflected to the upper left, and the loops of the intestine are retracted downward to the abdominal cavity to expose the inferior vena cava between just above the right renal vein and the point where the vena cava becomes buried in the liver. The vena cava in this region is freed from its connective tissue, and a ligature is passed under the vena cava.

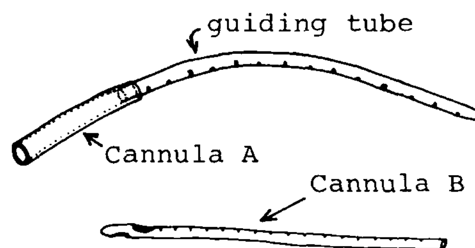

Figure 1—*Cannula A with a guiding tube and Cannula B. A guiding tube (polyethylene tubing, 0.2 cm o.d., 15 cm length) with its tip closed is inserted approximately 3 mm into Cannula A (polyethylene tubing, 0.2 cm i.d., 0.25 cm o.d., 3.2 cm length). The slight curve of the guiding tube is helpful in correcting false routes taken by the tip of Cannula A. Cannula A is filled with heparinized blood taken from another rat. Cannula B, with its tip closed, has two lateral holes near its tip. The guiding tube and Cannula B are put with centimeter markers throughout their length.*

The abdominal incision is then temporarily closed with two mosquito forceps. The right external jugular vein is exposed between the cephalic vein and the posterior external jugular vein and is ligated approximately 3 mm proximal to the junction of the posterior external jugular vein and the right external jugular vein. A small cut is made with scissors on the jugular vein, approximately 5 mm proximal to the ligature, while the jugular vein between the cut and the root of the cephalic vein is closed with a clamp to prevent bleeding.

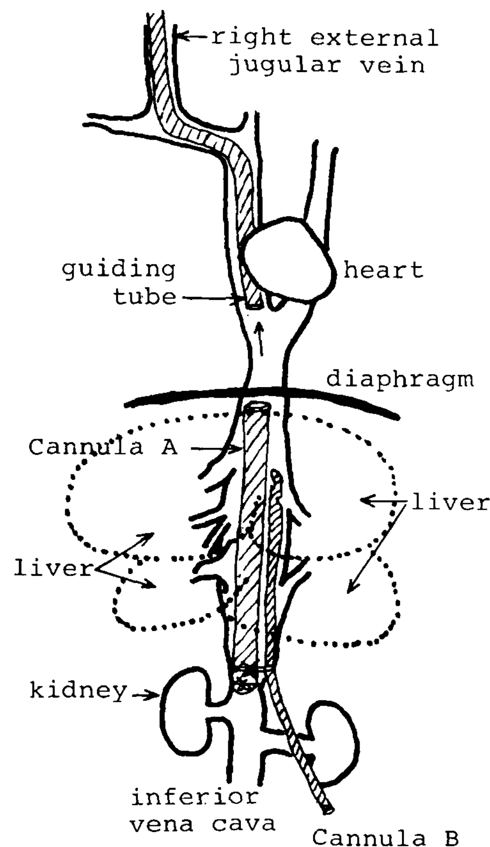

Figure 2—*Schematic representation of the inferior vena cava double cannulation for sampling mixed hepatic blood.*