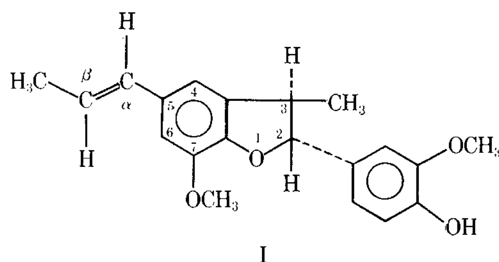

I

EXPERIMENTAL[5]

Air-dried stems, leaves, flowers, and fruits of *A. taliscana* (5.5 kg) were ground in a Wiley mill and macerated with ethanol–water (1:1 v.v). The aqueous ethanol extract was filtered and air dried, and the residue (1 kg) was repeatedly extracted with methanol until the methanol was clear. The combined methanol filtrate was air dried, and the residue was exhaustively extracted with ether. The combined ether extract, after removal of the solvent under vacuum, was subjected to three-funnel partition between benzene–methanol–water (8:5:1 v/v) (each phase was 1600 ml).

The lower phases were combined and air dried, and the residue (28 g) was chromatographed on an aluminum oxide (Grade III; 1 kg) column using hexane with increasing concentrations of acetone (0.1–0.5%) as the eluent. Fifty 250-ml fractions were collected. Based on TLC analysis, fractions 9–34 were combined to give a residue. Treatment with petroleum ether yielded a colorless material, which was filtered and then crystallized from methanol as colorless needles. The melting point, 130°, was undepressed by admixture with a synthetic sample.

The IR (KBr: λ_{max} 3450, 3040, 2970, 2930, 2880, 2850, and 955 cm^{-1}), UV [CH_3OH: λ_{max} 243 (log ϵ 4.55) and 276 (4.35) nm], NMR [$CDCl_3$: δ 1.35 (3H, d), 1.83 (3H, d), 3.12–3.73 (1H, m), 3.38 or 3.85 (3H, s), 3.38 or 3.85 (3H, s), 5.07 (1H, d), 5.67 (1H, s), 6.01–6.27 (1H, m), 6.01–6.27 (1H, m), and 6.73–6.93 (5H, m) ppm], and mass [*m/e* 326 (M^+, base), 311, 283, 202, 189, 163, 151, 149, and 137] spectra are in accord with Structure I. This compound was optically inactive, $[\alpha]_D^{24} \pm 0°$.

Anal.—Calc. for $C_{20}H_{22}O_4$: C, 73.68; H, 6.80; mol. wt., 326. Found: C, 73.70; H, 6.91; *m/e* 326 (M^+).

[5] Carbon and hydrogen analyses were carried out by Chemalytics, Inc., Tempe, Ariz. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. UV and IR spectra were run on a Beckman KB spectrophotometer and a Beckman IR-33, respectively. NMR and mass spectra were run using a Varian T-60 spectrometer and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. Optical rotations were run in chloroform using a Cary 60 spectropolarimeter.

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Rapid GLC Determination of Propranolol in Human Plasma Samples

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Abstract ☐ A rapid GLC method for measuring plasma propranolol levels is reported. 4-Methylpropranolol was utilized as an internal standard. Pentafluoropropionate derivatives of propranolol and the internal standard eluted rapidly and gave good sensitivity under the conditions employed. The advantage of this procedure over previously reported methods is the speed of analysis, which is facilitated by rapid elution of contaminant peaks.

Keyphrases ☐ Propranolol—GLC analysis, human plasma ☐ GLC—analysis, propranolol in human plasma ☐ Cardiac depressants—propranolol, GLC analysis in human plasma

Recent studies demonstrated that the effect of propranolol is plasma level related (1, 2). The accurate measurement of plasma propranolol levels has become essential for studies involving the mechanism of action of this agent; in some institutions, the monitoring of plasma propranolol levels is being used to aid patient therapy. Because of the increasing demand for rapid and accurate analysis of propranolol in plasma samples, previously reported procedures are being modified to increase efficiency and new procedures are being offered as improved alternatives.

The fluorometric method for measuring propranolol in biological fluids recently was reported to be limited with respect to both specificity and sensitivity (3). Three GLC procedures were proposed, two utilizing trifluoroacetyl derivatives (4, 5) and one utilizing a difluorobutyrate derivative (6); each employs a different internal standard. These three methods have their subtle differences and each, in order of publication, offers an advantage over the previous method. When analyzing large numbers of samples, however, these methods share one common disadvantage: the slow elution of contaminant peaks, which limits the frequency with which samples can be injected. Because of this limitation, an alternative procedure was developed, affording a greater efficiency by facilitating the more rapid analysis of large numbers of samples.

EXPERIMENTAL

Reagents—Glass-distilled[1] benzene, ethyl acetate, and cyclohexane were used, and 5 *N* NaOH, 0.2 *N* H_2SO_4, and 0.05 *M*, pH 11.5 phosphate buffer were prepared with double-distilled water and extracted twice with benzene. GLC grade pyridine[2] was diluted to 1.5% in ethyl acetate.

[1] Burdick & Jackson Laboratories, Muskegon, Mich.
[2] Pierce Chemical Co., Rockford, Ill.

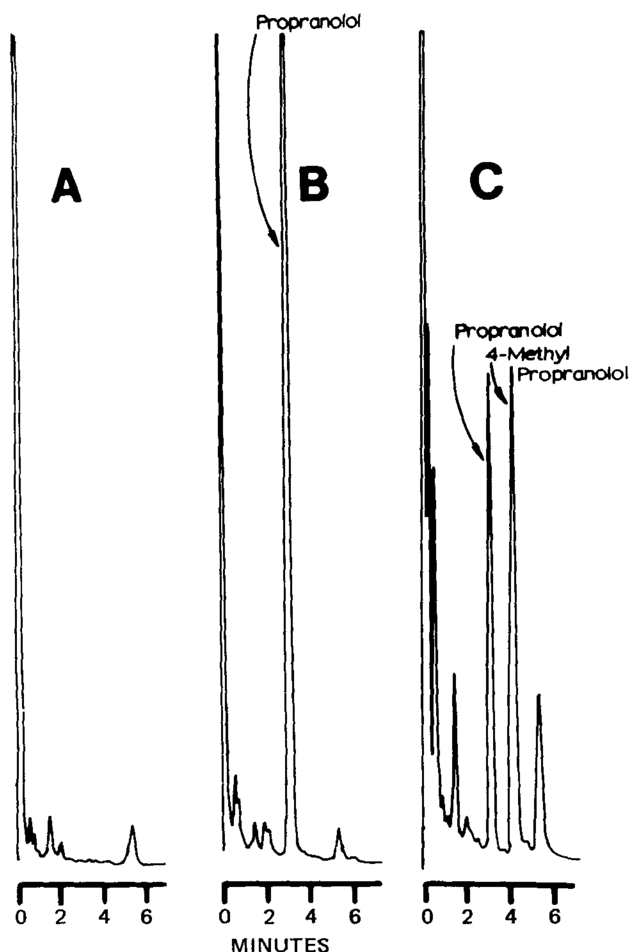

Figure 1—*Chromatograms of blank plasma (A), plasma from a patient taking propranolol (40 mg every 6 hr) (B), and plasma from a patient taking propranolol with 4-methylpropranolol added (C). Each chromatogram was prepared following injection of 3 μl at an attenuation of 256.*

Pentafluoropropionic anhydride[3] was the derivatizing agent, and 4-methylpropranolol[4] was the internal standard. Propranolol[5] was obtained as the hydrochloride salt.

GLC—A gas chromatograph[6] with a ^{63}Ni-electron-capture detector was used. A glass column, 180 cm × 2 mm i.d., was packed with 10% OV-1 on 80–100-mesh Gas Chrom Q[7]. The carrier gas was 5% methane in argon[8], and a flow rate of 20 ml/min was maintained. Analyses were performed under the following conditions: injection port temperature, 250°; column oven temperature, 245°; and detector temperature, 300°. A reporting integrator[9] was used for all data analysis.

Procedure—To 1 ml of plasma were added 200 ng of internal standard (20 μl of an aqueous solution), 3 ml of benzene, and 0.1 ml of 5 *N* NaOH. This mixture was shaken in polytef-coated screw-capped tubes for 5 min and then centrifuged. The benzene layer was transferred to a clean tube containing 1 ml of 0.2 *N* H_2SO_4. This tube was capped and shaken for 2 min and then centrifuged. The benzene layer was removed and discarded.

To the acidic layer was added 3 ml of clean benzene and 0.2 ml of 5 *N* NaOH. The tube was then capped and shaken for 5 min and centrifuged. The benzene was transferred to a clean tube and evaporated to dryness under nitrogen[10]. To the residue was added 0.5 ml of cyclohexane. The tube was then vortexed for 20 sec to redissolve the residue. Aliquots of 25 μl of 1.5% pyridine and pentafluoropropionic anhydride were then added.

[3] P.C.R. Inc., Gainesville, Fla.
[4] ICI 45,749, Imperial Chemical Industries, Macclesfield, England.
[5] Ayerst Laboratories, Rouses Point, N.Y.
[6] Hewlett Packard model 5736A.
[7] Applied Science Laboratories, State College, Pa.
[8] Matheson Gas Products, East Rutherford, N.J.
[9] Hewlett-Packard model 3380A.
[10] N-Evap, Organomation Associates, Shrewsbury, Mass.

Table I—Evaluation of Assay Precision and Accuracy (n = 7)

Concentration of Propranolol Added, ng/ml	Concentration of Propranolol Measured, ng/ml
10	11.2 ± 0.65 (*SD*)
25	27.9 ± 2.7 (*SD*)
50	52.3 ± 5.4 (*SD*)

The tube was capped, vortexed for 10 sec, incubated for 1 min at 70° on a dry bath heater, and then flushed with nitrogen to remove the excess pentafluoropropionic anhydride and to evaporate the cyclohexane. Cyclohexane, 50 μl, was then added, and the tube was vortexed for 10 sec. Phosphate buffer, 1 ml, was added, and the tube was shaken for 5 min and then centrifuged. Aliquots of 1–5 μl of the organic layer were then injected onto the column. All samples were analyzed in duplicate.

RESULTS AND DISCUSSION

Chromatograms of a blank plasma sample (A), a plasma sample from a patient taking propranolol (40 mg every 6 hr) with no internal standard added (B), and a patient plasma sample with internal standard added (C) are shown in Fig. 1. In the chromatogram of the blank plasma, there are no contaminant peaks with retention times near those of propranolol or the internal standard. In chromatogram C, the retention times of propranolol and the internal standard are 3.2 and 4.2 min, respectively. All three chromatograms were made at an attenuation of 256 following injection of a 3-μl sample.

Chromatogram B shows that none of the metabolites of propranolol, which are produced following oral administration of the drug, interferes with the measurement of the internal standard. Of the several propranolol metabolites identified following oral administration to humans (7), 4-hydroxypropranolol is structurally the most similar to propranolol. This metabolite is the most likely one to interfere with an analysis of the parent compound. With a pure sample, a retention time of 3.7 min was identified for the 4-hydroxy metabolite under the conditions employed. However, due either to removal by extraction or decomposition, this metabolite cannot be analyzed by this procedure; no peaks with this retention time were observed in the plasma samples analyzed.

Standard curves were prepared by extracting known drug concentrations and measuring the area under the propranolol and internal standard peaks. The ratio of the propranolol to internal standard areas was then plotted as a function of the concentration added. As a general procedure, a standard curve from 0 to 50 ng of propranolol/ml with 70 ng of internal standard was used for analyzing samples with low propranolol concentrations. For high level samples, a standard curve from 100 to 500 ng of propranolol/ml with 200 ng of internal standard was prepared. This procedure has been used routinely to analyze patient samples with concentrations ranging from below 1 to more than 400 ng/ml.

The standard curves were linear over the two intervals employed. Only a minor daily fluctuation in slope was observed. A mean slope of 0.284 ± 0.012 was found when five different low level standard curves were averaged. All standard curves passed through the origin. The precision and accuracy of this procedure were evaluated by adding measured amounts of propranolol and 70 ng of the internal standard to 21 samples of normal plasma. The samples were then extracted and analyzed (Table I). The percent standard deviation ranged from 6.2% at the low level to 10.3% at the higher level.

The extraction procedure used here is the same as previously published by Walle (4). The derivatization procedure, using pentafluoropropionic anhydride with pyridine as the catalyst, went to completion in less than 1 min under the experimental conditions. Once the samples were derivatized, they were stable for several days, and it was feasible to leave derivatized samples overnight for injection the following morning. However, once the samples were evaporated to dryness prior to derivatization, they had to be derivatized immediately. For unknown reasons, if the residue was left underivatized for any length of time, poor reproducibility occurred.

The final step of shaking the derivatized drugs with pH 11.5 buffer was necessary to hydrolyze contaminant substances. If the tubes were not shaken for this 5-min period, numerous slowly eluting peaks were encountered. The only significant peak eluting after propranolol was apparently due to a contaminant in the pentafluoropropionic anhydride.

This procedure facilitates the analysis of at least twice as many samples per day as with previously tried chromatographic procedures.

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Alterations Induced in Distribution and *In Vivo* Metabolism of Imipramine by Pregnenolone-16α-carbonitrile

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Abstract □ Female rats were given pregnenolone-16α-carbonitrile (I) to investigate its *in vivo* effects on the loss of the righting reflex and the mortality rate induced by imipramine as well as the concentrations of this drug and its metabolite, desipramine, in plasma, brain, liver, lungs, and kidneys. The protective action of I was associated with diminished organ levels of imipramine (catatoxic mechanism), and the relationship between brain and plasma imipramine concentrations remained unaltered. Desipramine–imipramine molar ratios were increased, indicating an elevated rate of *N*-demethylation. The unbound imipramine in plasma was diminished, but the relationship between protein-bound and unbound imipramine levels was not modified.

Keyphrases □ Pregnenolone-16α-carbonitrile—effect on pharmacological activity, distribution, and *in vivo* metabolism of imipramine, rats □ Imipramine—effect of pregnenolone-16α-carbonitrile on pharmacological activity, *in vivo* metabolism, and distribution, rats □ Distribution—imipramine, effect of pregnenolone-16α-carbonitrile, rats □ Metabolism—imipramine, effect of pregnenolone-16α-carbonitrile, rats □ Steroids—pregnenolone-16α-carbonitrile, effect on pharmacological activity, distribution, and *in vivo* metabolism of imipramine, rats □ Antidepressants—imipramine, effect of pregnenolone-16α-carbonitrile on pharmacological activity, distribution, and *in vivo* metabolism, rats

The protection afforded by catatoxic steroids against intoxication of diverse etiology, such as analgesics, carcinogens, and pesticides (1), was associated with induction of liver microsomal enzymes, particularly NADPH-dependent mixed function oxidases (2, 3), as well as with decreased levels of these toxicants in plasma, brain, and other tissues (3–7). Pregnenolone-16α-carbonitrile (I), a well-known hepatic microsomal enzyme inducer devoid of any other hormonal or pharmacological properties, exerts the greatest prophylactic effect *in vivo* (1) of the steroids studied.

At present, imipramine is the most frequently used tricyclic antidepressant. Its main metabolic pathways in the body include *N*-demethylation, *N*-oxidation, hydroxylation, and glucuronidation (8–12). Desipramine, one principal metabolite of imipramine, is pharmacologically active (13–15). Both imipramine and desipramine are lipophilic and pass into the brain, their target organ (16).

In view of the importance of imipramine in therapeutics, it was of interest to determine: (*a*) whether a drug-metabolizing enzyme inducer, *e.g.,* I, would produce characteristic alterations in drug distribution; (*b*) whether the changes in plasma drug concentration would be reflected in the brain as well as in the overall pharmacological effect; and (*c*) whether I would modify the influence of plasma protein binding on drug distribution.

EXPERIMENTAL

Female Charles River rats[1], 160–200 g, were maintained on laboratory chow[2] and tap water *ad libitum.* Compound I[3] was given twice daily for 3 days at a dose level of 68.3 mg/kg po in 0.5 ml of water as a micronized suspension homogenized with a trace of polysorbate 80. For comparative purposes, control animals received water with a trace of polysorbate 80. Imipramine hydrochloride[4], 80 mg/kg ip, was administered to all rats in 1 ml of distilled water once on the 4th day, 18 hr after the last pretreatment, unless otherwise stated.

Tables I and II indicate the various time periods at which blood samples were withdrawn under light ether anesthesia by aorta puncture into a syringe containing 0.2 *M* sodium oxalate. Tissues were removed and frozen until required for analysis. Drug-free plasma and tissues were used for preparing standards and blanks.

GLC—Imipramine and desipramine concentrations were measured by GLC[5]. A 1.5-m × 6.4-mm o.d. glass column packed with 1.5% OV-17 on 80–100-mesh Gas Chrom Q was used. The initial column, injector, and detector temperatures were 240, 300, and 300°, respectively. The column temperature was programmed for increases of 32°/min, starting 1 min after the time of injection. The final column temperature (280°) was maintained until 10 min postinjection. The nitrogen flow rate (as the carrier gas) was 18 ml/min, and hydrogen and compressed air pressures

[1] Canadian Breeding Farms & Laboratories Ltd., St. Constant, Quebec, Canada.
[2] Purina Laboratory Chow.
[3] The Upjohn Co., Kalamazoo, Mich.
[4] Ciba-Geigy (Canada) Ltd., Dorval, Quebec, Canada.
[5] Pye Unicam 104 chromatograph equipped with a flame-ionization detector and a Honeywell Electronik 194 Lab-Test recorder coupled with a disk integrator.