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Gas Chromatographic Separations of Sapogenins

Sir:

Gas chromatographic techniques have recently been developed for steroid separations. Suitable conditions have been found for the chromatography of a variety of steroids including plant and animal sterols, sex hormones, bile acids, adrenal cortical steroid hormones, steroidal amines and provitamins D_2 and D_3.[1] These methods may be used in many ways, but they are particularly useful for work with complex mixtures of natural origin. Among the naturally occurring steroids not previously investigated are the sapogenins; this group has now been studied. In addition to a spiroketal system, these compounds have a varying degree of substitution with hydroxyl and keto groups.

The conditions selected for a study of sapogenin separations involved use of a nonpolar phase (silicone polymer SE-30) at 225°. All of the compounds gave single, well defined peaks with no evidence of decomposition. The relative (to cholestane) retention times are in Table I. A sample of tigogenin recovered after chromatography was found to be unchanged (infrared comparisons) and repeated chromatography of the sample showed no indication of thermal alteration. Figure 1 shows the behavior of several sapogenins under these conditions.

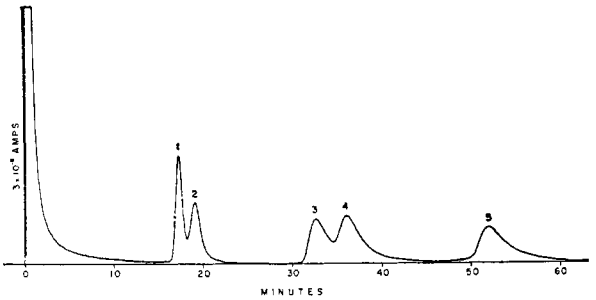

Fig. 1. Gas chromatographic behavior of (1) smilagenin, (2) tigogenin, (3) gitogenin, (4) chlorogenin, and (5) mexogenin. The conditions are those described for Table I

TABLE I[a]

Compound	C_{22}[b]	C_5[c]	Substituents	Time[d]
Sarsasapogenin	neo	β	3β-OH	2.57
Smilagenin	iso	β	3β-OH	2.47
Yamogenin	neo	Δ^5	3β-OH	2.66
Diosgenin	iso	Δ^5	3β-OH	2.64
Tigogenin	iso	α	3β-OH	2.71
Yuccagenin	iso	Δ^5	2α,3β-$(OH)_2$	4.68
Gitogenin	iso	α	2α,3β-$(OH)_2$	4.81
Chlorogenin	iso	α	3β,6α-$(OH)_2$	5.40
Hecogenin	iso	α	3β-OH 12-keto	4.96
Mexogenin	iso	β	2β,3β-$(OH)_2$ 12-keto	7.82
Manogenin	iso	α	2α,3β-$(OH)_2$ 12-keto	8.76
Kammogenin	iso	Δ^5	2α,3β-$(OH)_2$ 12-keto	8.33
Cholestane				1.00[e]

[a] Conditions: Column, 6 ft. × 5 mm.; 0.75% SE-30 polymer on 100–140 mesh Gas-Chrom P; 225°; 14 p.s.i.; argon ionization detector. [b] The configurations are 25L or neo and 25D or iso. [c] The notation refers to 5-H. [d] Relative to cholestane. [e] Time, 7.0 min.

Several correlations between structure and relative retention times may be seen from the data in Table I. The introduction of an additional substituent group (hydroxyl or carbonyl) leads to a relatively large increase in the retention time, and consequently the extent of substitution may be recognized from the chromatographic behavior. Compounds with differing ring A/B relationship were separated; the 5α-H isomer had a longer retention time than the 5β-H compound (compare tigogenin and smilagenin). Compounds with a Δ^5 structure gave retention times different from those observed for the corresponding saturated compounds. The effect of a change in configuration of the methyl group at C-25 was relatively small; in two examples the *iso*-series showed the lower retention times. The relatively small effect observed here parallels previous observations on the behavior of C-25 epimers in the steroidal amine series.

These results suggest that gas chromatographic techniques may be useful in studies of the occurrence, structure, and reactions of sapogenins. A number of steroid hormones are synthesized from

(1) W. J. A. VandenHeuvel, C. C. Sweeley, and E. C. Horning, *J. Am. Chem. Soc.*, **82**, 3481 (1960) (steroids); W. J. A. VandenHeuvel, C. C. Sweeley, and E. C. Horning, *Biochem. Biophys. Res. Comm.*, **3**, 33 (1960) (sex hormones and bile acids); W. J. A. VandenHeuvel and E. C. Horning, *Biochem. Biophys. Res Comm.*, **3**, 356 (1960) (adrenal cortical steroid hormones); W. J. A. VandenHeuvel, C. C. Sweeley, and E. C. Horning, *Separation of Steroids by Gas Chromatography*, Symposium on Drugs Affecting Lipid Metabolism, Milan, Italy, June 2–4, 1960 (sterols and sterol esters); C. C. Sweeley and E. C. Horning, *Nature*, **187**, 144 (1960) (steroids); W. J. A. VandenHeuvel, E. C. Horning, Y. Sato, and N. Ikekawa, *J. Org. Chem.*, in press (steroidal amines); E. O. A. Haahti, W. J. A. VandenHeuvel and E. C. Horning, *J. Org. Chem.*, in press (polyester phases for steroids); H. Ziffer, W. J. A. VandenHeuvel, E. O. A. Haahti, and E. C. Horning, *J. Am. Chem. Soc.*, in press (Vitamins D_2 and D_3); R. K. Beerthius and J. H. Recourt, *Nature*, **186**, 372 (1960) (sterols).

sapogenins, and the possibility now exists of monitoring these steroid transformation reactions by gas chromatography. The techniques should be equally useful in biochemical or biological studies involving sapogenins and other steroids.

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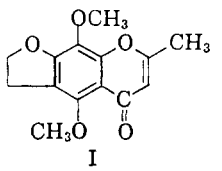
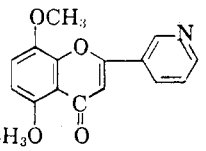
New Classes of Active Central Nervous System Depressing and Stimulating Agents

Sir:

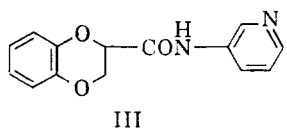
Khellin (I), a principal active component isolated from the fruit of the Mediterranean plant, *Ammi visnaga*, has been shown to possess a direct relaxing action on visceral smooth muscle and has been used frequently in the treatment of coronary deficiency disease.[1] A few synthetic 5,8-dimethoxychromones, which may be regarded as defuro-khellin derivatives, appeared recently in animal testing to have greater activity than khellin itself.[2] Also, it is of interest to note that one of the chief actions of many of the psychotherapeutic agents currently used in medical practice is to induce muscular relaxation[3] both in animals and humans. In the light of these facts, it was thought that some 5,8-dimethoxychromones, with selected substituents in the 2 position might have similar physiological effects.



I



II



III

Since late 1952 we have been working with the 5,8-dimethoxychromones and analogs.[4] Among many compounds of this type synthesized, one, 5,8-dimethoxy-2-(3-pyridyl)chromone(II), was found to be the most active as a central nervous system depressant. To our knowledge this is the first demonstration that a chromone possesses this type of activity.

Condensation of 2,5-dimethoxy-6-hydroxyacetophenone[5] with ethyl nicotinate in the presence of sodium hydride gave 3,6-dimethoxy-2-nicotinylacetophenol as bright yellow needles m.p. 120–121°. (*Anal.* Found for $C_{16}H_{15}NO_5$: C, 63.93; H, 5.02; N, 4.76). Treatment of this material with sulfuric acid in ethanol resulted in ring closure to yield the desired product (II) as pale yellow to colorless needles, m.p. 178–179° (*Anal.* Found for $C_{16}H_{13}NO_4$: C, 67.80; H, 4.63; N, 4.76).

Oral administration of II (50 mg./kg.) to mice greatly reduced spontaneous motility and increased evipal sleeping time from four to five times, while meprobamate, one of the most widely used psychopharmacologic agents, was needed in much larger doses (110–200 mg./kg.) in order to exhibit an equal effect. Also, the ataractic suppression of II against aggressive behavior in mice required a dose of 70 mg./kg. instead of 200 mg./kg. for meprobamate.[6] These tests indicated that II is about three times as potent as meprobamate. Meanwhile, II was found to be three times less toxic than that of meprobamate. Similarly, a very potent tranquilizing action was observed[7] by administration of II to fighting fish, cats, dogs and monkeys.

In exploring variations in the structure of II, it was recalled that the 1,4-benzodioxan ring system has a formal structural relation to that of chromone. Therefore, an extended series of compounds of this type was prepared.[8–10] *N*-(3-Pyridyl)-1,4-benzodioxan-2-carboxamide (III), m.p. 154–155° (*Anal.* Found for $C_{14}H_{12}N_2O_3$: C, 65.47; H, 4.80; N, 10.88), obtained by the acylation of 3-amino-pyridine with 1,4-benzodioxan-2-carbonyl chloride,[8] has been found[7] for the first time to exhibit outstanding central nervous system stimulating action as an analep-

(1) G. V. Anrep *et al.*, *J. Pharm. and Pharmacol.*, **1**, 164 (1949) and *Am. Heart J.*, **37**, 531 (1949); K. Samaan *et al.*, *J. Pharm. and Pharmacol.*, **1**, 538 (1949); E. N. Silber *et al.*, *J. Clin. Invest.*, **30**, 1046 (1951); L. A. Nalefski *et al.*, *Circulation*, **5**, 851 (1952); R. C. Kory *et al.*, *Am. Heart J.*, **50**, 308 (1955).

(2) G. Jongebreur, *Arch. intern. pharmacodynamic*, **90**, 384 (1952).

(3) For drugs belonging to this category see New and Nonofficial Drugs, evaluated by A.M.A. Council on Drugs, page 676 (1960).

(4) The first paper of this series not including compounds reported here is in press [J. Koo, *J. Org. Chem.*, in press].

(5) This compound was prepared by modification of the method of W. Baker (*J. Chem. Soc.*, 1922 (1939)] in four steps from 2,6-dihydroxyacetophenone, which in turn was obtained in another four steps according to the general direction of A. Russel and J. R. Frye [*Org. Syntheses*, Coll. Vol. III, 282 (1955)]. However, the first-step product, 4-methyl-7-hydroxycoumarin, was synthesized by following an improved procedure of J. Koo [*Chem. & Ind.*, 445 (1955)].

(6) C. Y. Yen, R. L. Stanger, and N. Millman, *Arch. intern. pharmacodynamic*, **123**, 179 (1960); L. Cook and E. Weidleg, *Federation Proc.*, **19**, 22 (1960).

(7) The pharmacology study by Dr. S. Krop and his associates, Mrs. M. Graeme and Mrs. E. Siegmund, of the pharmacology department, Ethicon, Inc., is gratefully acknowledged; the details will be published by them later.

(8) J. Koo, *et al.*, *J. Am. Chem. Soc.*, **77**, 5373 (1955).

(9) J. Koo, *et al.*, *Chem. & Ind.*, 832 (1958).

(10) J. Koo, *J. Org. Chem.*, in press.