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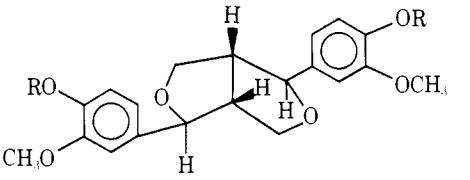
Isolation and Synthesis of Pinoresinol Diglucoside, a Major Antihypertensive Principle of Tu-Chung (*Eucommia ulmoides, Oliver*)

Sir:

Tu-Chung (*Eucommia ulmoides, Oliv.*) is one of the oldest herbs known and its medicinal value has been noted for several thousand years in China.[1] Tung Chung extract has long been known as a tonic for old people who can apparently drink it daily as tea without ill effects. Oral administration of Tu-Chung bark tea or wine to hypertensive patients showed that improvement occurred after 2–4 months in 93.6% of 62 cases of hypertension. Several investigators have confirmed the hypotensive action of aqueous and ethanol extracts of Tu-Chung bark in anesthetized dogs, cats, rabbits, rats, and guinea pigs.[2-5] The systemic arterial hypotension caused by the Tu-Chung extract is apparently the result of peripheral vasodilation by its direct action on the vascular smooth muscle.[5] Although much had been done on the study of the chemical composition of leaves and bark of *E. ulmoides*,[6] there has not been a systematic study of the pharmacologically active principles of this drug. We herein report the identification and synthesis of pinoresinol di-β-D-glucoside as the major antihypertensive principle of Tu-Chung bark.

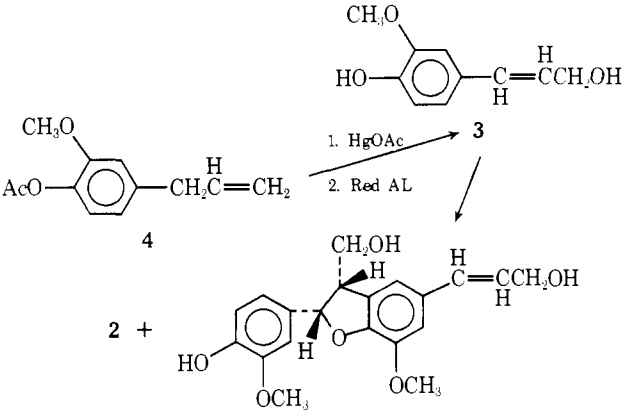
The antihypertensive activity was measured by the fall in the arterial blood pressure in anesthetized hypertensive rats.[7] Four successive chromatographies of the 95% ethanol extract of Tu-Chung bark (4.75 kg) over silica gel (MN-Kieselgel Brinkmann) columns using chloroform:methanol:water as eluent afforded 2.2 g of a glycoside, **1**: mp 221–230 °C; $[\alpha]^{25}D$ −27.3° (*c* 0.54, H_2O); uv (H_2O) 276 nm (ε 6750), 226 (ε 21 500). Anal. Calcd for $C_{32}H_4O_{16}\cdot 4H_2O$: C, 50.92; H, 6.68. Found: C, 51.23; H, 6.70.

Hydrolysis of **1** with β-glucosidase[8] (Sigma) afforded 2 mol of glucose, characterized by paper chromatography (ethanol:H_2O:1-butanol, 1:5:4) and oxidation with glucose oxidase, and an aglycone, **2**: mp 158–159 °C; molecular ion at *m/e* 358.141 63 (theory 358.141 10); its NMR and infrared spectra were found to be identical with those of an authentic specimen of (+)-pinoresinol.[9] As **2** is devoid of optical activity, it is apparent that the glycoside (**1**) consists of (±)-pinoresinol (**2**) linked to two D-glucose residues via β-glucosidic bonds.



1, R = D-glucose
2, R = H

Although two chemical syntheses[10,11] of (±)-**2** were reported, neither of these is applicable to the preparation of **2** in quantities sufficient for in-depth pharmacological evaluation. Syringaresinol may be efficiently prepared either by the incubation of syringin with crude emulsin[12] or by the exposure of 4-hydroxy-3,5-dimethoxycinnamyl alcohol to the action of mushroom laccase.[13] Unfortunately, when coniferin and coniferyl alcohol[14] (**3**) were, respectively, used as substrates in these enzyme systems, the major product formed was dehydrodiconiferyl alcohol,[15] and only traces of **2** were detected. On the other hand, the chloroperoxidase[16]-containing microorganism, *Caldariomyces fumago*, catalyzed the dimerization of coniferyl alcohol, prepared by oxidation of eugenol acetate (**4**) with mercuric acetate,[17] to (±)-pinoresinol. In a typical experiment, when 1 g of coniferyl alcohol (**3**) was exposed to *C. fumago*[18] for 16 h, 115 mg of (±)-pinoresinol, mp 158–159 °C, accompanied by 123 mg of (±)-*cis*-dehydrodiconiferyl alcohol[19] (**5**), mp 160–161 °C, was formed.



Reaction of (±)-**2** with α-bromoacetoglucose,[20] in the presence of Ag_2O, followed by alkaline hydrolysis, afforded **1** (50%) as a mixture of α,β-anomers, mp 232–235 °C; $[\alpha]^{25}D$ −33.5° (*c* 0.57, H_2O); its infrared, NMR spectra and antihypertensive activity[21] were found to be indistinguishable from those of **1**, obtained from *E. ulmoides*.

Isolation and characterization of other minor biologically active components are currently in progress and will be reported later.

Acknowledgments. The authors express their appreciation to Dr. Paul K. T. Sih for bringing this problem to our attention, and to Drs. John Rowe and John Harkin for supplies of (+)-pinoresinol. This research was supported by grants from the Wisconsin Alumni Research Foundation, the National Institutes of Health under Grant AM-4874, and the Miles Laboratories.

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(22) Each value given represents a single rat.

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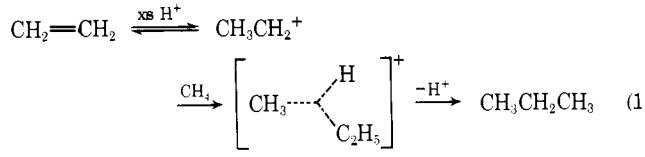
Strong Acid Chemistry. 3.[1] Alkene–Alkane Alkylations in HF–TaF_5. Evidence for the Presence of $C_2H_5^+$ in Solution

Sir:

Selective acid catalyzed alkylations of ethylene and propylene by the lower alkanes, methane, ethane, and propane, have never been clearly experimentally demonstrated, although the ability to carry out these reactions has been claimed.[2-5] The thermodynamics for these reactions to occur catalytically are very favorable below ~225 °C. Above this temperature antagonistic entropy effects become more important. Below this temperature acid catalyzed cleavage products from competing olefin oligomerization reactions must be distinguished from the simple alkylation products.

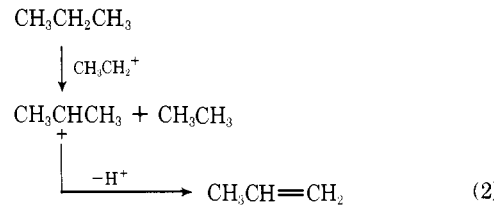
Olah discovered that the lower alkanes could be ionized at 50 °C and indeed could participate in further self-condensation (alkylation) reactions in antimony pentafluoride containing strong acid systems.[6] The door was opened to new chemistry through this activation of traditionally passive small molecules.[7-10]

We felt that a logical approach to achieve the catalytic reaction of methane would be to react it with a very energetic primary carbenium ion. The simplest way to generate such an ion is to dissolve ethylene, at moderate temperature in an excess of a strong acid. The ion would thus be available to react with the strongest base available, i.e., methane, in an alkene–alkane alkylation. This is in sharp contrast with the traditional alkane–alkene alkylations.[11] We have now found that such simple addition reactions can be selectively carried out in the HF–TaF_5 catalyst system. A methane–ethylene (85.9%:14.1%) gas mixture was passed at a rate of 42 standard cm^3/min through a 300-cm^3 Hastelloy C Autoclave Engineers autoclave containing 50 cm^3 of a 10:1 HF–TaF_5 (2.0 mol/0.20 mol) system stirred at 1000 rpm at 40 °C and maintained at 40 psig. In order to assure maximum protonation of the ethylene and minimize possible competition from ethylene oligomerization reactions a 40-fold excess of acid as well as efficient mixing was maintained and the temperature was not permitted to vary more than ±1°. Gas samples were taken from a system installed in the exit line and analyzed on a Perkin-Elmer Model 900 gas chromatograph using an 18 ft Silica Gel–10 ft DC-200 column connected in series and a flame ionization detector. After both 1.5 and 2.5 h the total C_3 in the reaction product amounted to 58% (eq 1). Mechanistically,



(1)

the ethyl cation appears to directly alkylate methane via a pentacoordinated carbonium ion such as proposed by Olah. It should be noted that methane alone does not react with HF–TaF_5 under these conditions[12] and that unless a flow system is used, the propane product, which is a substantially better hydride donor than methane, reacts further with the intermediate ethyl cation to ultimately form ethane and propylene in equal amounts (eq 2). Only ~1% of the propane formed in the flow system reacts with another molecule of methane to form isobutane. Also, based upon the results of acid quenching and analysis of hydrocarbons, only traces of isopentane and isohexanes, and no heavier materials, were present in the acid. No hydrogen could be detected in the product with a thermal conductivity detector.



(2)

A less favorable mechanistic pathway is one in which an ethyl cation abstracts a hydride ion from a methane molecule to form a methyl cation (less stable than $C_2H_5^+$ by 39 kcal/mol).[13] The methyl cation can then alkylate a molecule of ethylene to produce propyl$^+$, etc. This alternative can be ruled out because the ethane thus formed includes a hydrogen needed to form propane product catalytically, and would consequently lead to increased formation of propylene and/or polymeric products. In an attempt to generate primary cations and to simulate the ethylene–methane alkylation, ethyl chloride was reacted with methane under alkylation reaction conditions. When no propane or propylene product was observed the reaction of methyl chloride with ethane was carried out. These latter two reactions[14] proceed without any involvement of the alkane and provide evidence that the ethylene–methane alkylation proceeds through a stabilized species such as a pentacoordinated carbonium ion. By this we mean a species having one three-centered two-electron bond, not a carbon having five directly bound ligands (see eq 1).[15] It should also be noted that propane formation, as a degradation product of polyethylene, can be ruled out because ethylene alone, diluted in helium, reacts, under these conditions, with no propane formation. Under similar reaction conditions, but in a hydrogen atmosphere, polyethylene (mol wt 20 000) reacts quantitatively with 10:1 HF–TaF_5 to form C_3–C_6 alkanes, with isobutanes and isopentanes constituting 85% of the product. Results of the polymer reaction are best understood in terms of known