The synthesis of other C_{21}-methyl steroids including the 6α-fluoro analog of V will be described in a subsequent report.

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Zusammenfassung

Die Darstellung der beiden epimeren C_{21}-Methylderivate des 9α-Fluorprednisolons wird beschrieben. 9α-Fluor-21-methyl-1,4-pregnadien-11β,17α,21B-triol-3,20-dion-21-azetat ist ein hochwirksames entzündungshemmendes Steroid, vollkommen frei von Nebenwirkungen der Natrium- und Wasserretention des C_{21}-unsubstituierten 9α-Fluorprednisolons.

Phenol-Mineral Interactions: The Oxidation of Pyrogallol and Other *o*-Diphenols on Silica Gel[1]

Recently, HESS, BACH, and DEUEL discussed evidence for the formation of complexes between *o*-diphenols and various Si-O-Si-compounds, offering them as models for the interactions leading to soil organo-mineral substances[2]. Experimentally, they demonstrated the dissolution of silicagel, alumina, and other minerals in ammoniacal catechol with the formation of crystallizable complexes or compounds. Their highly significant experiments were carried out under comparatively vigorous temperature conditions. It may also be of significance that phenol-mineral interactions take place under mild conditions. Thus, the enzymic oxidation of eugenol to lignin-like polymers is enhanced by highly ordered minerals such as the Amphiboles and Serpentines[3]. Even in the absence of enzyme, chrysotile, hornblende, and muscovite can catalyze (at 25°C) the oxidative polymerization of eugenol to water-insoluble substances which can be eluted from the mineral surface with organic solvents[4].

In the presence of chrysotile, granite or hornblende, the oxidation of catechol at ordinary temperatures led to formation of NaCl-precipitable polymers in 23–32% yield. In their absence, one-tenth as much polymer was formed. The products formed in the presence of different minerals were distinguished by spectroscopic and chemical analysis.

Chrysotile, granite and garnet catalyzed ring hydroxylation and oxidation in phenylalamine at 60°C in the presence of H_2O_2.

In other experiments, glass fibres have been shown to catalyze pyrogallol oxidation in ethanolic solution[5].

Although these systems were set up as models for surface-catalyzed phenol oxidations and differ in approach from the work reported by HESS, BACH, and DEUEL, they may be of value in revealing other aspects of the phenol-mineral interaction.

Experimental. Silicagel[6] was washed three times with hot 10% HCl (1 l/g of gel), soaked in deionized water for 6 h and rinsed in running, de-ionized water for 1 h. The washed product contained no spectrophotometrically detectable impurities, and gave negative tests for Fe (III) and Cu (II).

When M/10 pyrogallol in water or ethanol was poured over a column of washed silicagel, a yellow-brown product formed on the gel surface in less than 1 sec. The product continued to darken on the gel surface and in the supernatant for an additional 10–20 min. The adsorbed pigment was difficultly soluble in alcohol, incompletely soluble in water, but soluble in 10% HCl.

The effect of small quantities of silicagel was followed by photometric determination of oxidation product at 425 mμ. In 50 ml of M/10 KH_2PO_4, 5 *mM* of pyrogallol were dissolved and silicagel added in varying amounts. After they were shaken at 25°C for 1 h, the absorbancies of supernatants and HCl-eluates were determined. The following activity was observed for a 50-mesh gel which had been dried to constant weight over P_2O_5 at 35°C before use:

Silicagel (mg)	Absorbancy at 425 mμ		
	Supernatant	Gel	Total (as%)
0	0·062	—	100
60	0·067	0·019	139
120	0·073	0·021	150
300	0·083	0·056	224
600	0·092	0·085	287

In ethanol, pyrogallol autoxidizes at one-twentieth the rate observed in aqueous media, and the addition of 600 mg silicagel to 5 *mM* of pyrogallol in 50 ml 95% ethanol increased the rate of oxidation 30-fold.

Silica gel is also active in chloroform where pyrogallol autoxidation is nil. Even air-dry silica gel mixed with pyrogallol powder develops a black surface deposit during several weeks at 25°C.

When the gel is heated for 30 min at 500°C, its catalytic activity is lost. Similarly, fine β-quartz powder had no activity at all.

In addition to the commercial product, gels were prepared from sodium metasilicate and tetraethyl orthosilicate $(C_2H_5O)_4$ Si, using HCl or acetic acid. When M/100 HF was used, the gel formed from ethyl orthosilicate was virtually inactive.

The oxidation products formed under aqueous conditions were somewhat variable in composition, but yielded C 48–55%, H 1–2%, and ash 1–2%. These products gave no definite melting point, were lightened somewhat in color by reducing agents, and reacted vigorously with bleaching on addition of peracetic acid. Their ultraviolet absorption characteristics resemble closely those described for catechol polymers formed in the presence of mineral surfaces[4].

Other phenols were also tested as silicagel substrates. Phenol, hydroquinone resorcinol, caffeic acid and chlorophenols were not oxidized, whereas the *o*-diphenols catechol, gallic acid, and dihydroxyphenylalanine (DOPA)

[1] Most of this work was carried out at the University of Rochester with the support of Grant C-2730, National Cancer Institute, U. S. Public Health Service.

[2] R. HESS, R. BACH, and H. DEUEL, Exper. *16*, 38 (1960).

[3] S. SIEGEL, J. Amer. chem. Soc. *79*, 1628 (1957).

[4] S. SIEGEL, Proc. Nat. Acad. Sci. U. S. *43*, 811 (1957).

[5] S. SIEGEL, F. PORTO, and P. FROST, Arch. Biochem. Biophys. *82*, 330 (1959).

[6] Fisher Scientific Co. Silica gels, up to 200 mesh (gas chromatography grade). The ethyl silicate used was a Fisher purified grade, and sodium metasilicate was a certified reagent grade.

[7] The work of ZIECHMAN and KROLL cited in [2]. – F. SCHEFFER and W. KROLL, Agrochimica *4*, 97 (1960).

like pyrogallol were converted into brown or red-brown products rapidly.

The catalytic activity of the gel was markedly reduced by 0·01–01 m Al(III), Ca(II) and Mg(II). Hydroquinone, resorcinol, and halgenated monophenols also inhibited pyrogallol oxidation on the gel. Methylene blue which is strongly adsorbed by silicagel also seemed inhibitory whereas eosin which has little affinity for silicagel also failed to suppress its catalytic activity.

Discussion. The experiments which have been described demonstrate the silicagel activated oxidation of *o*-diphenols. Accordingly, they provide evidence for the formation of complexes between silicagel and these phenols, as do earlier investigations cited.

The importance of hydrogel structure and surface charge, in catalysis are indicated by heat inactivation, the inactivity of β-quartz, and the inhibitory effects of cations. Phenolic inhibitors suggest that hydrogen bonding may also be one of the significant interactions between substrate and catalyst.

The specificity of silicagel activation (as distinct from adsorption alone) for *o*-diphenols parallels the silicate-coordinating properties of these compounds. In both systems catechol, pyrogallol and gallic acid were active, whereas phenol, hydroquinone, and resorcinol were not. Although some inorganic material is carried into solution the conditions described in this report clearly do not favor complete or extensive silicon-oxygen bond scission. Nevertheless it is likely that catalysis involves the coordination of *o*-diphenols at the silicagel surface. The oxidation products are in part firmly adsorbed on the gel, but are released by acid, perhaps by decomposition of surface complexes.

These findings support the phenol-mineral interactions and mineral-catalyzed phenol oxidations[7], which have been proposed as factors in the formation of humic substances in soil.

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Zusammenfassung

Die Oxydation von Pyrogallol, Brenzkatechin, Gallussäure und Dopa wird von Silikagel, nicht jedoch von β-Quarz, katalysiert. Diese Katalyse erfolgt nicht nur in Wasser, sondern auch in wässerigem Alkohol und Chloroform. Dabei scheinen die elektronegativen Oberflächen des Silikagels von Bedeutung zu sein. Mg^{2}, Ca^{2+} und Al^{3+} sowie verschiedene Phenole, die keine *o*-Dihydroxygruppierung besitzen, hemmen die katalytische Oxydation.

Von den geprüften Phenolen sind nur bei *o*-Diphenolen Komplexbildung mit Si^{4+} und katalytische Oxydation durch Silikagel festzustellen.

Die Bedeutung von Phenol-Mineral-Komplexen bei der Bildung von Humusstoffen wird durch die vorliegenden Untersuchungen gestützt.

A Differential Staining Reaction Demonstrating Reciprocal Activity in Mauthner's Cells[1]

In an attempt to relate the structure of the neuron with its function, extensive neurohistological and neurophysiological studies are being made on the bilaterally represented Mauthner's cells which lie in the floor of the medulla oblongata of teleosts. It was proposed[2,3], that these 2 cells function as reciprocating units so that while one cell is actively excited the other is actively inhibited. This functional relationship fulfills the requirements of a system demonstrating reciprocal inhibition of motor neurons by the sensory afferents.

It was also noted[2] that in some histological preparations of the Mauthner's cells of the bullhead there was a differential staining of the various parts of these neurons (dendrites, cell body, and axon hillock region). It was suggested that this staining reaction might be indicative of specific intracellular changes characterizing neuronal excitation as well as inhibition.

This report is concerned with a further attempt to determine the effects of controlled stimulation of the VIIIth nerve afferents on the staining reaction of the paired Mauthner's cells of the bullhead.

Materials and Methods. Fifteen bullheads (10 experimental and 5 controls) were used. Under hypothermic anesthesia, craniotomy exposed the VIIIth nerve roots and facilitated rapid removal of the brain for processing by freeze-dehydration. In the experimental fish the entering roots of the VIIIth nerve were stimulated at threshold level (1 sec at 10/sec). This was done on the right side of 5 fish and on the left side of the other 5. The brain was removed immediately and processed. The same procedure was followed on the 5 control specimens except that no stimulus was applied to the nerve roots.

Tissues frozen in Isopentane (– 100°C) are dehydrated for 7 h in vacuum apparatus at pressure of not more than 1·0 μ and temperature – 40°C. Dehydrated tissues embedded in paraffin are sectioned serially and mounted on glass slides. Staining is similar to the classical Bodian protargol technique[4] except reduction is carried out using the Pearson-O'Neill silver gelatin[5]. Deparaffinized sections, after hydration in 3 changes distilled water, 2 min each, are placed in freshly prepared 0·5% aqueous protargol with a 2 × 2 inch copper plate in the glass container. Incubate in 37°C oven 18–24 h. Wash 10 min in running water, 2 changes of distilled water, 5 min each, and one change of buffered[6] water for 2 min. Reduce in silver gelatin[7] mixture for 30 sec. Wash in 4 changes of distilled water, 5 min each. Tone in 0·2% gold chloride 2 min. Wash in 2 changes distilled water, 5 min each. Place in 2% oxalic acid for 15 sec, wash in 2 changes distilled water, 5 min each, and finally treat in 5% sodium thiosulfate, 2 changes, 5 min each. Wash in running water for 5 min, dehydrate and cover.

Results. The typical staining reaction of the Mauthner's cell on the same side as the applied VIIIth nerve stimulus is characterized by dendrites and perikaryon which are colored a deep purple while the axon hillock and the proximal part of the axon is an almost translucent lavender (Fig. 1). In contrast, the Mauthner's cell contralateral to the applied stimulus stains in a reverse manner with the dendrites and cell body colored lavender and the axonal

[1] Supported in part by the NIH Grants MY-3271 and H-3084.

[2] E. Retzlaff, J. comp. Neur. *101*, 407 (1954).

[3] E. Retzlaff, J. comp. Neur. *107*, 209 (1957).

[4] D. Bodian, J. comp. Neur. *68*, 117 (1937).

[5] A. Pearson and S. L. O'Neill, Anat. Rec. *95*, 297 (1946).

[6] Buffered water is prepared as follows: 17 ml of 5 *M* acetic acid (12 ml to 1000 ml distilled water) added to 3 ml of sodium acetate (16 g to 1000 ml distilled water). Buffered water prepared by adding 14 ml of stock buffer to 1000 ml distilled water. The pH is approximately 4.1.

[7] The silver gelatin reducing solution, using buffered water, is prepared as follows: (1) 400 ml 3% gelatin heated to 70°C; (2) 100 ml 2% $AgNO_3$; (3) 40 ml 1% hydroquinone. Stir (2) and (3) together and add to (1) immediately before reducing sections.