sponding to about 1/60 ml of urine) was similarly treated.

As standards 100 μg of oestrone were taken through the procedure in the presence of 1 ml of urine from a non-pregnant mare. All urine samples and standards were developed in duplicate.

The optical densities were read at the wavelength of maximal absorption of oestrone, 533 mμ, and also 33 mμ to both sides. The corrected optical densities calculated as $E_c = 2E_{533} - (E_{500} + E_{566})$ were assumed to be linearly related to the oestrogen concentration.

Fluorimetry of the methylene chloride extracts was performed with a Beckman model DU monochromator supplied with an attachment made in the laboratory (to be published) and a photomultiplier combination. As incident light an unfiltered beam of a wolfram continuum was used. The light emitted at a right angle of the incident beam was recorded at 550 mμ (maximal emission of oestrone) and at 520 mμ. The slit of the monochromator was kept at 0.1 mm corresponding to a half intensity band width of about 2.5 mμ. The difference between the light intensities at the two wavelengths was assumed to be due to oestrogens.

The results of colorimetric and fluorimetric analyses are plotted against each others in Fig. 1. It may be seen that the values of the oestrogen concentration obtained with both photometric methods were in good agreement. Estimates of the precision [2] of the fluorimetric method in different ranges of urinary oestrogen concentration are given in Table 1. It is evident that oestrogen concentrations below 10—20 μg per ml could not be determined with any reasonable confidence. It is questionable to which degree positive values in this range represent oestrogens. At very low oestrogen concentrations the net fluorescence was always positive whereas the corrected optical densities were sometimes negative. Colorimetry at low concentrations tended to give erratic results apparently due to the relatively high unspecific (background) absorption and inadequacy of the correction formula applied.

During the months 5 and 6 after breeding the ratio between oestrone and equilin, which apparently are the major oestrogens excreted in the pregnant mare, seems to be high, but may decrease towards parturition [3]. The results of the method described for the estimation of total urinary oestrogens would be influenced by any variations in this ratio since the chromogenic (and fluorigenic) value of equilin was much lower than that of oestrone.

The results reported indicate that the amounts of oestrogens being excreted in the pregnant mare are subject of large variations. Due to its practicability the crude method described might be of some value for further studies of these variations and of the possible variations of the time after breeding at which an appreciably increased excretion of oestrogens is initiated.

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Isothiocyanates XLVI *. Glucocappasalin, a New Naturally Occurring Thioglucoside

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Capparis salicifolia Griseb. *(Capparidaceae)* is a tree indigenous to the Chaco region of the Argentine. Paper chromatography of an extract of its seeds** revealed the presence of two new thioglucosides with R_B-values [1] of 0.81 and 1.17 in butanol:ethanol:water (4:1:4). We here wish to report on the structure of the fastest migrating glucoside for which the name *glucocappasalin* is proposed.

After purification by ion exchange, counter-current separation of the two glucosides (380 transfers), and acetylation, *glucocappasalin tetraacetate* was obtained

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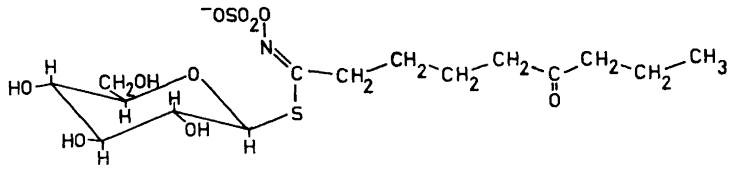

I

as a crystalline potassium salt, m.p.* 133–136° (decomp.), $[\alpha]_D^{22}$ −16.0° (*c* 1.0, H_2O). λ_{max}^{MeOH} 223 mμ (ε 6490) (shoulder at 210 mμ). IR-spectrum (KBr): very similar to that of glucocapangulin tetraacetate [2] with a sharp band at 1700 cm^{-1}, indicative of a keto-grouping. Analytical data** agreed with the composition $C_{23}H_{34}O_{14}NS_2K,0.5\ H_2O$ for the acetate. Assuming the general thioglucoside structure to be present, this formula leaves $(C_7H_{15})(CO)$ to be accomodated as the side-chain.

Ethanolysis, catalyzed by pyridinium ions[3], converted the acetate into *desulphoglucocappasalin tetraacetate*, $C_{23}H_{35}O_{11}NS$, m.p. 144°, $[\alpha]_D^{21}$ −16.1° (*c* 2.0, CH_3OH), λ_{max}^{MeOH} 213 mμ (ε 5100), IR_{KBr}:keto band at 1695 cm^{-1}. The NMR-spectrum*** (in $CDCl_3$) exhibited, besides the expected signals from a β-glucopyranoside tetraacetate moiety, a highly shielded (τ 9.12) triplet (J = 7 cps) corresponding in area to three protons and, hence, attributable to *one* CH_3-group located next to a CH_2-group and without double bond functions in β-position. The combined NMR-data clearly demonstrated the presence in glucocappasalin of an *unbranched* C_8-chain containing a C=O-grouping in γ, δ or ε-position relative to the terminal methyl group.

On acid hydrolysis glucocappasalin or its tetraacetate afforded, as expected [4], besides sugar, hydroxylamine and sulphate, an oxononanoic acid, characterized as the semicarbazone, m.p. 150°. On mass-spectroscopic analysis* the methyl ester of the oxo-acid could be unequivocally identified as 6-oxononanoic acid methyl ester (molecular ion peak at *m/e* 186, strong peaks at *m/e* 154, 155 (M−32, M−31), 143 (M−$CH_3(CH_2)_2$), 111 (143−32), 71 ($CH_3(CH_2)_2CO$)). On critical comparison with synthetic specimens of the semicarbazone of 6-oxononanoic acid, m.p. 150° (lit. values: 154−5° [5], 153° [6]), and the methyl ester[7] (identical IR and mass spectra), this identification was confirmed.

Hence, the glucocappasalin ion is represented by structure (I)**, which is a higher homologue of glucocapangulin previously encountered in *Capparis angulata* Ruiz et Pav. [2], a close botanical relative of *C. salicifolia* Griseb. The presence in (I) of an unbranched C_9-skeleton renders its biogenesis intriguing.

A full account of the present work will appear in a forthcoming paper.

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* Melting points are uncorrected. Heating rate: 1°/min.

** Satisfactory analyses and consistent infrared spectra have been obtained for the new compounds reported.

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* The authors are much indebted to Dr. R. Ryhage, Stockholm, for performing the mass spectra.

** In the semi-systematic naming of thioglucosides recently proposed [3], glucocappasalin would be: potassium 5-oxooctyl glucosinolate.

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