The influence of the methoxyl group on the acidity and charge distribution in phenols is illustrated by a plot of the pK's of the substituted guaiacols against the pK's of the corresponding phenols without the methoxyl group (*cf.* Fig. 1). It is of interest to note that the correlation for *p*-substituted guaiacols and phenols is almost linear (*cf.* also the correlation between the values of the Hammett function σ and the infra-red hydroxyl spectra in Ref.[5]); only a slight increase in acidity (difference between dotted and full line in Fig. 1) with increasing electronegativity of the substituent is evident.

However, the relationships are much more complex with the *o*-substituted compounds. When strong intra-molecular hydrogen bonds are formed, *i.e.* when a $-NO_2$, $-CHO$ or $-COCH_3$ group is in *o*-position relative to the phenolic hydroxyl, the methoxyl group, through charge displacement in the unionized guaiacol molecule, decreases the ionization tendency and consequently the acidity. This effect increases rapidly with decreasing electronegativity of the chelating group, *i.e.* in the order 4,6-dinitro < *o*-NO_2 < *o*-CHO < *o*-$COCH_3$.

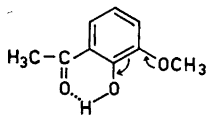

Fig. 2. Charge displacement in 2-methoxy-6-acetylphenol.

The strong effect of the *o*-acetyl group as compared with the formyl and nitro groups is especially noteworthy. In *o*-hydroxynaphthones the corresponding effect is markedly weaker[7], which, however, is attributed to the deviation of the acetyl groups from the plane of the ring, resulting in the weakening of conjugation and hydrogen bonding simultaneously. It is evident that the large decrease in acidity in *o*-hydroxyacetophenones is in some way caused by the electron-donating methyl group, but a valid explanation can be obtained only by quantum mechanical calculation of the electron distribution, *cf.* Ref.[8] In a later report these and related effects in phenolic compounds of the guaiacol type will be discussed with the aid of such calculations.

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1. Lindberg, J. J. *Soc. Sci. Fennica, Commentationes Phy.-Math.* **20** (1957) No. 1; **21** (1958) No. 7.
2. Juslén, C. and Lindberg, J. J. *Finska Kemistsamfundets Medd.* **68** (1959) 53; Lindberg, J. J., Nordström, C.-G. and Laurén, R. *Suomen Kemistilehti* **B 35** (1962) 185.
3. Albert, A. and Serjeant, E. P. *Ionization Constants of Acids and Bases*, London 1962.
4. Bates, R. G. *J. Res. Natl. Bur. Std.* **66 A** (1962) 179.
5. Lindberg, J. J. *Finska Kemistsamfundets Medd.* **69** (1960) 11.
6. Braude, E. A. and Nachod, F. C. *Determination of Organic Structures by Physical Methods*, New York 1955, p. 589; Ågren, A. *Acta Chem. Scand.* **9** (1955) 49; *cf.* also Magnusson, L. B., Postmus, C., Jr. and Craig, C. A. *J. Am. Chem. Soc.* **85** (1963) 1711.
7. Bisanz, T. and Dybowska, B. *Roczniki Chem.* **33** (1959) 975.
8. Enebäck, C. *Soc. Sci. Fennica, Commentationes Phy.-Math.* **28** (1963) No. 10.

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Preparation of Pure Thyroglobulin

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For the study of thyroid proteins a new method for the purification of thyroglobulin was developed. Both hog and human thyroid tissue was used. The hog thyroid was obtained from the local abattoir and the human thyroid tissue from non toxic goitre patients by thyroidectomy. In one case radioactive iodine was administered 72 h before the operation.

The thyroid tissue was frozen at −20° and cut into slices about 1 mm thick. The slices were suspended in cold saline solution and left overnight at −4°. After shaking the solution was centrifuged and the supernatant brought to a volume of 15 ml

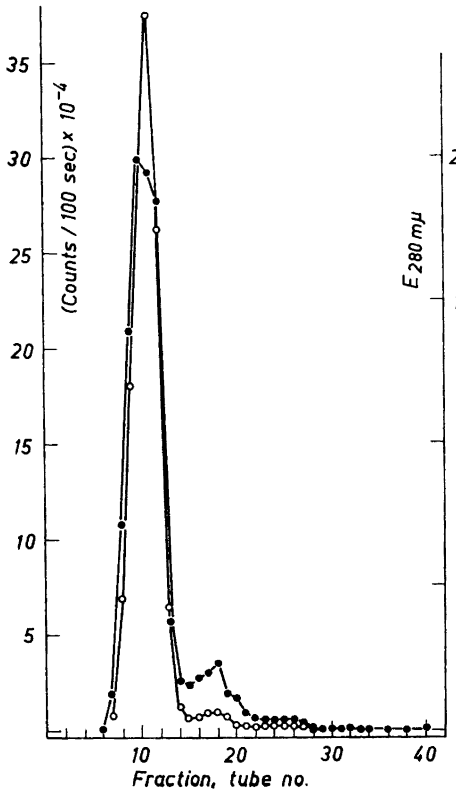

Fig. 1. Fractionation of the saline extract of radioactive human thyroid tissue on a Sephadex G-200 column. Solid circles = absorption at 280 mμ, open circles = radioactivity.

by ultrafiltration. The concentrated solution was applied on a Sephadex G-200 (Pharmacia, Uppsala, Sweden) column (2.8 × 50 cm). Elution was carried out with a phosphate-NaCl buffer solution (0.15 μ, pH 6.5, NaCl 0.05 M). The effluent was collected by means of a fraction collector. The radioactivity and the optical density (at 280 mμ) were determined. The thyroglobulin peak (Fig. 1) was separated and rechromatographed after having been brought to a volume of 5—10 ml by ultrafiltration.

The thyroglobulin solution was next applied to a DEAE-cellulose column[1] (Schleicher & Schüll; 2.1 × 30 cm). First elution was carried out using a 0.15 μ phosphate-NaCl buffer solution overnight and the effluent was discarded. Further elution was made with another phosphate-NaCl buffer solution (0.40 μ, pH 6.5, NaCl 0.30 M) and the fractions were collected with a fraction collector. The fractions corresponding to the peak of optical density and of radioactivity were pooled and subjected to ultrafiltration. The thyroglobulin solution was then dialysed for 24 h against a phosphate-NaCl buffer (0.40 μ, pH 6.5).

Ultracentrifugation of both hog and human thyroglobulin revealed only one peak with the calculated $s°_{20\,w}$ values of 18.8 and 19.3, respectively. When human thyroglobulin was diluted in the proportion 1:2 with a 0.40 μ phosphate-NaCl buffer another slower component appeared. This component amounted to not more than 0.5 % of total protein and had an estimated $s°_{20\,w}$ value of about 12.5. Its appearance was evidently due to the dilution.[2]

Immunoelectrophoresis of the human thyroid homogenate against anti-human serum produced 4 precipitation bands none of which contained radioactivity on autoradiography. After purification these bands disappeared. Immunoelectrophoresis of human thyroid homogenate against a human serum having a high anti-thyroglobulin titer (haemagglutination titer 1:2.5 mill.)

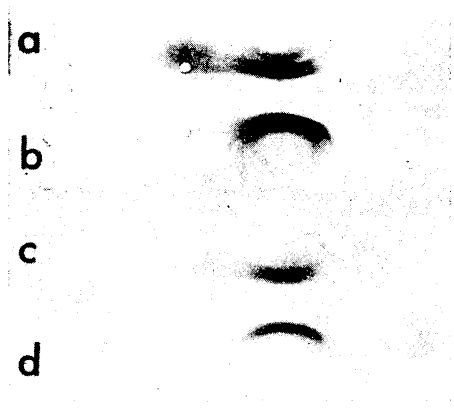

Fig. 2. Immunoelectrophoresis of radioactive human thyroid protein against "anti-thyroglobulin" serum.
Protein staining of a) thyroid homogenate, b) purified thyroglobulin.
Autoradiography of c) thyroid homogenate, d) purified thyroglobulin.

revealed precipitation of some radioactive compounds apart from the thyroglobulin. These compounds were, however, absent after purification of the thyroglobulin (Fig. 2). The purified hog thyroglobulin produced, however, no precipitation band with this anti-thyroglobulin containing human serum.

This method which combines fractionation on Sephadex G-200 column and the previously used purification on DEAE-cellulose offers some advantages as compared to other methods. Our own studies indicate a much more satisfactory preliminary purification with Sephadex than with ammonium sulphate hitherto used.

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1. Ui, N. and Tarutani, O. *J. Biochem. (Tokyo)* **50** (1961) 508.
2. Edelhoch, H. *J. Biol. Chem.* **235** (1960) 1326.

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Synthesis of 2-Phenylisopropylhydrazine-1-^{14}C Hydrochloride

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Interest in organic hydrazine compounds is largely a result of their more or less pronounced inhibiting action upon the enzyme monoamine oxidase.[1,2] Recently, it has also been observed that certain hydrazine derivatives possess cytostatic properties,[3-6] possibly due to the low release of hydrogen peroxide known to occur when hydrazines undergo autoxidation.[6,7]

2-Phenylisopropylhydrazine is one of the most potent monoamine oxidase inhibitors and has been used widely as an antidepressant drug. In order to make studies on the metabolism and distribution of this compound in biological systems possible a method for the synthesis of ^{14}C labelled 2-phenylisopropylhydrazine has been developed. The method should be generally applicable to the synthesis of ^{14}C labelled hydrazine derivatives of this type.

The synthesis includes reaction steps (1)–(5) (R = phenyl, R′ = CH_2CH_2–$CH(NH_2)COOH$):

$$RCH_2MgCl + {}^{14}CO_2 \rightarrow RCH_2{}^{14}COOH \quad (1)$$

$$RCH_2{}^{14}COOH + CH_3Li \rightarrow \underset{\text{I}}{RCH_2{}^{14}CCH_3} \quad (2)$$

$$RCH_2{}^{14}CCH_3 + NH_2NHCOR' \rightarrow$$

$$RCH_2{}^{14}C{=}N{-}NHCOR' \quad (3)$$
$$\quad\quad CH_3 \quad\quad \text{II}$$

$$\text{II} \xrightarrow{H_2/Pt} RCH_2{}^{14}CH{-}NHNHCOR' \quad (4)$$
$$\quad\quad\quad\quad CH_3 \quad\quad \text{III}$$

$$\text{III} \xrightarrow[HCl,100°]{\text{concd.}} RCH_2{}^{14}CH{-}NHNH_3{}^{+}Cl^{-} + R'COOH \quad (5)$$

Labelled phenylacetone (I, R = phenyl) has been synthesized previously by the reaction between phenylacetyl chloride and ethyl ethoxymagnesiummalonate and subsequent hydrolysis and decarboxylation of the intermediate malonic ester,[8] but this method was found to give consistently low yields of a rather impure product. Instead, phenylacetone was prepared by reaction of free phenylacetic acid with an excess of methyllithium,[9] which gives about 90 % yield of a pure product. This method avoids the troublesome step of preparing the acid chloride.

Step (3) involves the preparation of the solid γ-glutamylhydrazone of phenylacetone, (II), which can then be catalytically hydrogenated using Adam's catalyst without any side reactions resulting from cleavage of the nitrogen to nitrogen bond. Under these reaction conditions it is not possible to avoid this side reaction if the unsubstituted phenylacetone hydrazone is