The fluorimetric assay was as for 5-hydroxytryptamine (5-HT) (Andén & Magnusson, 1967). Fig. 1 shows typical spectra of 5-HTP in brain samples of mice treated with Ro 4-4602, 4 h before death.

The concentrations of 5-HTP in mouse brains at various times after Ro 4-4602, 800 mg/kg, are shown in Fig. 2. A rapid accumulation of 5-HTP was seen between 7·5 and 15 min after the injection of the decarboxylase inhibitor. The highest amounts were found after 2 h, about 0·3 μg/g brain. After 4 h the values had declined, probably because of the short duration of action of the inhibitor. The accumulation of 5-HTP between 7·5 and 15 min (about 0·1 μg/g) corresponds to a synthesis rate of 5-HT of 0·8 $\mu g/g\ h^{-1}$. Other authors have reported a turnover rate of 5-HT in rat brain of 0·3 $\mu g/g\ h^{-1}$ (Diaz, Ngai & Costa, 1968). Whether this difference represents a real discrepancy, remains to be elucidated.

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The quantitative analysis of alkyl polyoxyethylene glycol monoethers with mass spectrometry and proton magnetic resonance spectroscopy in combination

The most recent spectroscopic method to be applied to the analysis of non-ionic surfactants is proton magnetic resonance (pmr) spectroscopy which, apart from qualitative data, has the added advantage of providing quantitative data without the use of standard compounds, provided a suitable internal standard, like the aromatic protons of a polyoxyethylene alkylphenol, is present in the molecule.

We now report the use of a combination of pmr spectroscopy and mass spectrometry in the examination of the purity of samples of dodecyl tetra-, hexa- and octa- oxyethylene glycol monoethers ($C_{12}E_4$, $C_{12}E_6$ and $C_{12}E_8$, respectively) which were prepared (Corkill, Goodman & Ottewill, 1961) for experiments reported elsewhere (McDonald, 1969). The mass spectra of these samples showed, as expected, molecular ions at m/e = 362, 450 and 538, respectively, and the expected fragmentations by stepwise loss of CH_2 and ($O{\cdot}CH_2{\cdot}CH_2$) units, indicated by a series of peaks at $M^+ -14$, $M^+ -28$, $M^+ -42$, etc. and $M^+ -44$, $M^+ -88$, etc., respectively, in each spectrum. Small peaks at m/e = 376 ($M^+ +14$) (13% of the molecular ion peak) and at m/e = 390 ($M^+ +28$) (5% of the molecular ion peak) were present in the spectrum of $C_{12}E_4$, indicating the presence of homologous impurities ($C_{12}E_4 + CH_2$) and ($C_{12}E_4 + 2CH_2$), respectively, in the sample. Similarly, small peaks at m/e = 464 ($M^+ + 14$) (1% of the molecular ion peak) and at m/e = 478 ($M^+ +28$) (5% of the molecular peak) were present in the spectrum of $C_{12}E_6$, indicating the presence of impurities of molecular formula ($C_{12}E_6 + CH_2$) and ($C_{12}E_6 + 2CH_2$), respectively, whereas the spectrum of $C_{12}E_8$ had no peaks of

greater m/e than its molecular ion. Since there were no peaks other than those mentioned above in these mass spectra at higher m/e than the molecular ion peaks, there are no impurities in the samples containing additional ethylene oxide moieties. The possibility that the sample contained impurities with a lower number of ethylene oxide moieties cannot, of course, be ruled out from the mass spectral evidence alone. However, this spectral evidence coupled with the results of the classical method of analysis for ethylene oxide moieties (Siggia, Starke & others, 1958), which agreed with the nominal values for pure samples, make this unlikely. Consequently, we can now use the signal caused by the protons of the CH_2–O group in the pmr spectra of these samples as an internal molecular standard in a quantitative analysis of these compounds: this was not possible in earlier pmr measurements (Flanagan, Greff & Smith, 1963) where mass spectral data was not available.

The pmr spectra were recorded in deuterochloroform solution on a Varian A 60 spectrophotometer with tetramethylsilane as internal standard and checking each integral over three runs. From a table of spectra (Table 1) the combined integrals of

Table 1. *The proton magnetic resonance spectral data of* $C_{12}E_4$, $C_{12}E_6$ and $C_{12}E_8$.

Protons giving rise to signal	Sample $C_{12}E_4$ Signal (τ)	Sample $C_{12}E_6$ Signal (τ)	Sample $C_{12}E_8$ Signal (τ)
–O–$(CH_2)_2$–O	6·34 (s) } 26[a]	6·33 (s) } 27[a]	6·37 (s) } 26[a]
Dodecyl CH_2–O	6·57 (t) (J = 7Hz) }	6·53 (t) (J = 7Hz) }	6·57 (t) (J = 7Hz) }
Dodecyl C–CH_2–C	8·74 (s)[c] 30[b]	8·72 (s)[c] 21[b]	8·75 (s)[c] 15[b]
Dodecyl CH_3–C	9·13 (t) (J = 6Hz)	9·12 (t) (J = 6Hz)	9·12 (t) (J = 6Hz)
–OH	6·89 (s, broad)	6·98 (s, broad)	Not observable

[a] Combined integral. [b] Integral. [c] Strong singlet masks, but incorporates the quartet caused by the methylene protons of the ethyl group of the dodecyl moiety.

the low-field singlet and low-field triplet of $C_{12}E_4$, $C_{12}E_6$ and $C_{12}E_8$ (i.e. 26, 27 and 26, respectively) must correspond to 18, 26 and 34 protons, respectively (i.e. 2m + 2 where m is the number of methylene groups in the polyoxyethylene chain). Thus it can be seen readily that the corresponding integrals of the high-field singlet (i.e. 30, 21 and 15, respectively) are equivalent to 20·8, 20·2 and 19·6 protons ($\pm 2\%$ owing to errors in determining integral values), compared with 20 protons (i.e. 2n-2 where n is the number of methylene groups in the dodecyl moiety) which would be expected for pure compounds.

If in the above mass spectra $M^+ + 14$, $M^+ + 28$, etc. peaks were absent but $M^+ + 44$, $M^+ + 88$, etc. peaks were present, the high-field singlet in the pmr spectrum would then have acted as internal molecular standard, assuming lower homologues than dodecyl ethers were absent. If, however, the mass spectra indicated the presence of both series of peaks above M^+ then no internal molecular standard would be available (the OH and CH_3 signals are relatively too weak and ill-defined) and the above approach would not be valid.

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