# Method for the Determination of Molecular Weights

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*Summary* A simple method is illustrated for the accurate determination of molecular weight; the method is particularly applicable in antibiotic chemistry.

IN connection with other work it became necessary to develop a method for the determination of molecular weights of organic compounds, other than by mass spectrometry, and more accurate than the usual osmometric and similar methods.

If one forms a derivative of a compound with a radiolabelled reagent, the exact specific activity of which is known, its molecular weight can be calculated according to equation (1) or (2), where $M_X$ = molecular weight of unknown (as its derivative); $a_X$ = activity in $\mu$Ci/mg of

$$M_X \cdot a_X = n \cdot A_R \quad (1)$$

$$M_X = \frac{n \cdot A_R}{a_X} \quad (2)$$

unknown; $n$ = number of equivalents of reagent which have reacted with the unknown; $A_R$ = specific activity of reagent ($\mu$Ci/mmol).

Both $A_R$ and $a_X$ can be determined with a degree of accuracy limited only by the errors encountered in weighing and counting methods.

For volatile reagents, *e.g.* diazomethane, $A_R$ cannot be determined directly with the degree of accuracy needed. In that case, one forms a second derivative, this time with a known compound which then in turn gives $A_R$.

A major problem is the determination of $n$, *i.e.* the number of reactive substituents in the unknown. This can usually be done by a combination of other physical measurements, especially n.m.r.

The derivatives made have to be very pure. Since many high molecular weight compounds do not crystallize easily, we avoided crystallization altogether and purified all compounds tested by repeated t.l.c. followed by thorough drying.

In principle, considerably higher accuracy should be obtained and the development of the method is still under investigation.

*E.g.* pregnenolone [$^3$H]acetate $M$ 358) was prepared from pregnenolone (100 mg), and [$^3$H]acetic anhydride (0·2 ml) in pyridine (2 ml). The acetate was isolated and purified (t.l.c., 2 mm 8 × 8 silica gel plates). The material was eluted [$CHCl_3$–EtOAc (1 : 1)], the solvents were evaporated off, the residue was taken up in methylene chloride, and

inorganic matter removed by filtration and centrifugation. After freeze-drying for 48 h, a sample was weighed and counted on a liquid scintillation counter (Packard model 2003). $A_R = 11{\cdot}61\ \mu$Ci/mmol; $a_X = 0{\cdot}01628\ \mu$Ci/mg; $n = \frac{1}{2}$; $M_X = 356{\cdot}6$.

TABLE

| Compound | Molecular Formula | Molecular Weight Calc. | Molecular Weight Expt. |
|---|---|---|---|
| Pregnenolone acetate .. | $C_{23}H_{34}O_3$ | 358 | 356·6<br>363 |
| Everninocintriacetate[1] .. | $C_{31}H_{34}Cl_2O_{10}$ | 507 | 500 |
| Everninose-tetra-acetate[2] | $C_{22}H_{34}O_{14}$ | 522 | 517 |
| Megalomicin-triacetate[3] | $C_{50}H_{86}N_2O_{18}$ | 1002 | 995 |

The results of other test compounds are summarized in the Table.

A referee directed our attention to the method of molecular weight determination by double labelling.[4] The use of radioactive tracer techniques to determine the molecular weights of large molecules is well known. We have not, however, found it used in antibiotic chemistry where, we believe, it will have important applications.

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