The Constitution of Stravidin, a Novel Microbiological Product

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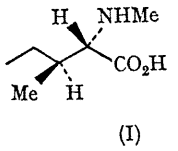
THE micro-organism *Streptomyces avidinii* produces an antibiotic, MSD-235, which is derived from a pair of components (MSD-235S and MSD-235L).[1] These components have been separated and although each of the pair is inactive they clearly interact synergistically because when they are combined the antibiotic complex MSD-235 is obtained which gives *in vitro* activity and *in vivo* protection against gram-negative micro-organisms. Streptavidin (MSD-235L) has already been identified as a high molecular weight protein and MSD-235S includes at least two related compounds (MSD-$235S_2$ and MSD-$235S_3$). We report here upon the novel constitution of the major component, MSD-$235S_3$, now called stravidin, and MSD-$235S_2$.

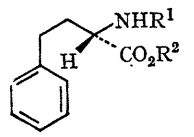
Acid hydrolysis of stravidin, $C_{17}H_{29}N_3O_3$,† yielded

† All new compounds have been characterised by analysis and by u.v., i.r., n.m.r., and mass spectra.

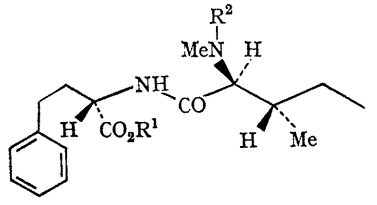
ammonia, (2*S*,3*S*)-*N*-methylisoleucine[2] (I), and (2*S*)-2-amino-4-phenylbutyric acid[3] (II) in equimolecular proportions. Stravidin is a monocarboxylic acid and with diazomethane gave an unstable methyl ester $C_{18}H_{31}N_3O_3$, which was transformed by atmospheric oxidation into dehydrostravidin methyl ester, $C_{18}H_{29}N_3O_3$.



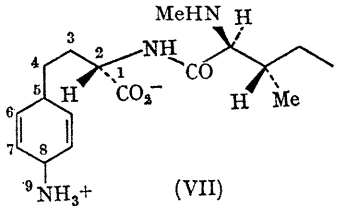
(I)



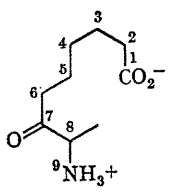
(II) $R^1=R^2=H$
(IV) $R^1=CO\cdot OCH_2Ph$, $R^2=H$
(V) $R^1=H$, $R^2=Bu^t$



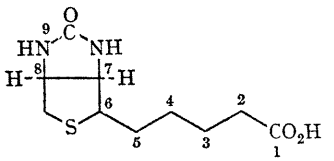
(III) $R^1=R^2=H$
(VI) $R^1=Bu^t$, $R^2=CO\cdot OCH_2Ph$



(VII)



(VIII)



(IX)

Spectroscopic examination of stravidin suggested the presence of a 4-substituted cyclohexa-2,5-dienylamine residue which provided the phenyl group in the hydrolysis product (II) and the *p*-aminophenyl group present in dehydrostravidin methyl ester. Stravidin, $C_{17}H_{29}N_3O_3$, when heated (100°, 1 hr.) in water, yielded ammonia and a transformation product, $C_{17}H_{26}N_2O_3$, which was characterised as a methyl ester. Consideration of these results in relation to the established constitutions of the hydrolysis products, (I) and (II), of stravidin suggested that the transformation product, $C_{17}H_{26}N_2O_3$, had the constitution (III), and this was confirmed by total synthesis.

N-Benzyloxycarbonyl-(2*S*)-homophenylalanine[4] (IV) was transformed by standard methods into the t-butyl ester (V) which was coupled with *N*-benzyloxycarbonyl-*N*-methylisoleucine by reaction with *NN'*-dicyclohexylcarbodi-imide in methylene chloride. The intermediate amido-ester (VI) with hydrogen bromide–acetic acid gave the compound (III), identical with the transformation product (III) obtained from stravidin. In order to account for the presence of a 4-substituted cyclohexa-2,5-dienylamine residue in stravidin, the zwitterionic formulation (VII) is now suggested. This constitutional formula (VII) for stravidin is fully compatible with detailed n.m.r. spectral and extensive high-resolution mass spectrometric studies on stravidin and its derivatives, which will be discussed in our full publication.

The transformation of stravidin (VII) into compound (III) is mechanistically analogous to (i) the transformation of santoninamine into hyposantonin[5] and (ii) the formation of hypoartemesin from artemesin oxime *via* the corresponding amine sulphate.[6] All these reactions take place under similar conditions.

Recently considerable interest has developed regarding the biological activity of a number of synthetic compounds containing the 1,4-cyclohexadiene system.[7] Stravidin is the first natural product to be recognised which contains a 4-alkylcyclohexa-2,5-dienylamine residue, and this novel constitution (VII) is of interest in several respects. It may be noted that there is evidence which suggests that stravidin can inhibit biotin synthesis by susceptible organisms.[1] It is already established that 8-amino-7-oxopelargonic acid has a vitameric relation to biotin (IX)[8] and a structural correlation (see numbers 1—9) between the 8-amino-7-oxopelargonic acid structure (VIII) and stravidin (VII) may be noted.

MSD-235S_2 has a constitution related to that of stravidin (VII) in which the *N*-methylisoleucyl residue is replaced by an *N*-methylvalyl residue.

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