Compound 1. TLC Si gel R_f 0.41 (EtOAc–Py–H_2O–MeOH, 80:12:10:5); cellulose 0.33 (15% HOAc). UV λ_{max}^{MeOH} nm: 271, 330; +NaOAc 277, 350; +NaOAc +H_3BO_3 273, 330; +$AlCl_3$ 279, 305, 348, 381; +$AlCl_3$ +HCl 280, 304, 344, 381; +NaOH N/10 281, 332, 397. Permethyl ether: MS (*m/e*) 660 (M^+, 17%), 645 (M−15, 24%), 629 (M−31, 100%), 541 (M−119, 20%), 529 (M−131, 21%), 515 (M−145, 12%); TLC Si gel R_f 0.21 ($CHCl_3$–EtOH–Me_2CO, 5:4:1), PM 6,8-di-*C*-α-L-arabinopyranosylapigenin 0.11, PM 6,8-di-*C*-β-D-xylopyranosylapigenin 0.30.

Compound 2. TLC Si gel R_f 0.49 (EtOAc–Py–H_2O–MeOH, 80:12:10:5); cellulose 0.31 (15% HOAc). UV λ_{max}^{MeOH} nm: 273, 331; +NaOAc 275, 302 (i), 341; +NaOAc+H_3BO_3 273, 333; +$AlCl_3$ 279, 306, 348, 384; +$AlCl_3$+HCl 277, 305, 344, 378; +NaOH N/10 280, 332, 396. Permethyl ether: MS (*m/e*) 660 (M^+, 16%), 645 (M−15, 26%), 629 (M−31, 100%), 541 (M−119, 20%), 529 (M−131, 28%), 515 (M−145, 14%); TLC Si gel R_f 0.26 ($CHCl_3$–EtOAc–Me_2CO, 5:4:1).

Compound 3. TLC Si gel R_f 0.58 (EtOAc–Py–H_2O–MeOH, 80:12:10:5); cellulose 0.15 (15% HOAc). Permethyl ether: MS (*m/e*) 660 (M^+, 24%), 645 (M−15, 25%), 629 (M−31, 100%), 541 (M−119, 30%), 529 (M−131, 58%), 515 (M−145, 26%); TLC Si gel R_f 0.21 ($CHCl_3$–EtOAc–Me_2CO, 5:4:1).

Compound 4. TLC Si gel R_f 0.78 (EtOAc–Py–H_2O–MeOH, 80:12:10:5), 0.65 (EtOAc–MeOH–H_2O, 63:12:9); cellulose 0.11 (15% HOAc). UV λ_{max}^{MeOH} nm: 269, 331; +NaOAc 274, 295, 354; +$AlCl_3$ 276, 304, 346, 384; +$AlCl_3$+HCl 276, 302, 342, 382; +NaOH N/10 277, 330, 392. Permethyl ether: MS (*m/e*) 486 (M^+, 91%), 355 (M−131 100%), 341 (M−145, 69%), 325 (M−161, 13%), 311 (M−175, 12%); TLC Si gel R_f 0.23 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4).

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FLAVONOIDS FROM THE LEAVES OF *CASSIA LAEVIGATA*

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Key Word Index—*Cassia laevigata*; Leguminosae; ombuin; quercetin 7,4′-dimethyl ether; ombuin 3-*O*-(2-rhamnosylglucoside).

Ombuin (3,5,3′-trihydroxy-7,4′-dimethoxyflavone) and a new glycoside, 3-*O*-(2-rhamnosylglucosyl)-ombuin, have been isolated from the leaves of *Cassia laevigata*. The flavonol was identified by mp, mmp, colour reactions, derivatization, UV, IR, NMR and MS. It has been reported earlier from many plant sources [1–3].

The glycoside gave the characteristic reactions of a flavonol glycoside [4] and the acid hydrolysate showed the presence of ombuin, glucose and rhamnose. Methylation and hydrolysis gave quercetin 5,7,3′,4′-tetra-*O*-methyl ether (mp, mmp, co-chromatography, UV and IR spectra) showing that the sugars were linked at position 3 of the aglycone. The NMR spectrum of the glycoside had a doublet at δ 1.32 which is typical of the rhamnose methyl group. The broad signal at δ 3.82, which overlapped with the methoxyl singlet at δ 4.00, was also due to the sugar protons. The nature of the sugar linkage was deduced by comparison of the rhamnose methyl signal with corresponding signals of neohesperidoside, δ 1.20 (*d*) and rutinoside, δ 0.80–0.95 (*br*) [5–7] to be of the neohesperidoside type. Thus it is identified as ombuin-3-*O*-neohesperidoside.

EXPERIMENTAL

The leaves of *Cassia laevigata* were obtained from United Chemicals and Allied Products, Calcutta-1, India. Dried ground leaves were extracted with EtOH and the conc extract diluted with H_2O to give a coloured ppt. which after column chromatography of the EtOAc-soluble fraction on Si gel gave ombuin, mp 229°.

The EtOAc extract of the original aq. soln gave three compounds (Si gel TLC; EtOAc–MeOH, 1:1) which were separated by column chromatography. The EtOAc eluate contained a single entity on TLC (Si gel, EtOAc), a yellow glycoside, mp 310° (d). IR ν_{max}^{KBr} cm^{-1}: 3370, 2920, 2860, 1625, 1530, 1485, 1180, 1160, 1140, 850, 830, 800, 760 and 690. UV λ_{max} nm: EtOH 255, 355; $AlCl_3$ 275, 419; $AlCl_3$–HCl 270, 415; NaOAc 256. 385: NaOAc–H_3BO_3 260, 365; NaOEt 275, 418. ^1H NMR (90 Hz, $(CD_3)_2SO_4$): δ 1.32 (3H, *d*, *J* = 12 Hz, rhamnose-Me), 4.00 (*s*, 6H, OMe), 4.90 (1H, *s*, H-1″ rhamnosyl); 5.20 (1H, *br*, H-1″

glucosyl), 3.85 (*br*, sugar protons), 6.42 (1H, *d*, $J = 2$ Hz, H-6), 6.80 (1H, *d*, $J = 2.5$ Hz, H-8), 7.67 (2H, *dd*, $J = 2.5$, 8.5 Hz, H-2′–H-6′); 6.95 (1H, *d*, $J = 8.5$ Hz, H-5′).

On acid hydrolysis the compound gave ombuin and chromatography of the aq. layer on Whatman No. 1 paper in EtOAc–Py–H_2O (12:5:4) and EtOAc–*i*-PrOH–H_2O (3:1:1) showed glucose and rhamnose. On methylation (Me_2SO_4–K_2CO_3) and hydrolysis, 5,7,3′,4′-tetramethylquercetin was identified by mp and co-chromatography with an authentic sample.

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EFFECT OF NICOTINE ON CAROTENOGENESIS IN EXTREMELY HALOPHILIC BACTERIA

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Key Word Index—*Halobacterium halobium*; *Sarcina litoralis*; *Amoebobacter morrhuae*; extremely halophilic bacteria; pigmented strains; morphologically different; nicotine; carotenoids; bacterioruberins.

Nicotine, first introduced by Howes and Batra in 1970 as a bioregulator of carotenogenesis in mycobacteria [1, 2], has since been reported to affect carotenogenesis in photosynthetic and non-photosynthetic bacteria [3–6], fungi [7, 8] and higher plants [9].

Recently we have found that nicotine had a pronounced effect on carotenogenesis, particularly of the C_{50} pigments in *Halobacterium cutirubrum* [10]. In this report we examine the effect of nicotine on carotenogenesis in three representative and morphologically different genera of extreme halophiles, i.e. *H. halobium* (rod shape), *Sarcina litoralis* (a halococcus) and *Amoebobacter morrhuae* (a highly pleomorphic form of the genus *Halobacterium*). Our results show that nicotine is a potent regulator of carotenogenesis in extreme halophiles, irrespective of their morphological differences.

When the above strains of extreme halophiles were grown in the presence of increasing concentrations of nicotine, the formation of bacterioruberin and monoanhydrobacterioruberin was completely inhibited by 1.0 mM nicotine (Table 1). Concomitantly, large increases in levels of lycopene and bisanhydrobacterioruberin were observed. The maximum accumulation of these two compounds occurred at 3 mM nicotine for *H. halobium* and *S. litoralis* and at 6 mM nicotine for *A. morrhuae*. The total carotenoid content of the three strains at the optimal concentrations of nicotine was usually 24–31% higher than that of control cultures (Table 1). Such a stimulatory effect of nicotine on total carotenoid production has also been observed in photosynthetic bacteria [4]. It is also noteworthy that growth of the above organisms is not inhibited in up to 6 mM nicotine, but at higher concentrations it is drastically reduced.

The results presented in this paper indicate that nicotine is a potent inhibitor of the C_{50} bacterioruberin and monoanhydrobacterioruberin and also of β-carotene in the genus *Halococcus* (e.g. *Sarcina litoralis*), in the rod-shaped halophiles of genus *Halobacterium* (e.g. *H. halobium*) and in *Amoebobacter morrhuae*, a highly pleomorphic halobacterium, as was reported previously for *Halobacterium cutirubrum* [10]. The present findings are also consistent with the view presented previously [10] that the C_{50} carotenoids may be formed from a C_{40} carotene, probably lycopene.

EXPERIMENTAL

Cultures of the above microorganisms were grown aerobically for 5 days at 37° in 1 l. batches of standard complex medium for halophiles in 4 l. shake flasks in an incubator shaker as described previously [11]. Appropriate amounts of nicotine were added, aseptically, at the time of inoculation as described elsewhere [10]. Cells were harvested, washed and estimated for protein content by the method of Lowry *et al.* [12]. Total lipids were extracted by the Bligh–Dyer procedure [13] and neutral lipids were separated by acetone precipitation of the total lipids as described previously [10, 11]. Individual carotenoids were purified by applying the acetone-soluble lipids to Si gel G TLC plates and developing in $CHCl_3$–MeOH (93:7) for bacterio-